Condensed tannin and cell wall composition in wine grapes: Influence on tannin extraction from grapes into wine

by

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ABSTRACT

Condensed tannins derived from the grape berry contribute to the organoleptic properties of wine, in particular, astringency, as well as wine colour and aging stability. The contribution of different grape tannin structures to wine quality is not well understood. In particular, the measurement of tannin in grapes is not indicative of the amount and type of tannin extracted into wine, which makes it difficult to predict the impact on wine quality. Tannin extraction is thought to be influenced by interactions between tannins and cell walls of the grape berry.

This study aimed to investigate the influence of grape tannin and cell wall composition on extraction of tannin into wine. Tannin distribution in terms of the distribution of polymer length or degree of polymerisation (DP), the concentration and subunit composition was determined in grape skin, seed and wine of Shiraz and Cabernet Sauvignon wine grapes. The polysaccharide composition and tannin binding capacity of cell walls and the amount of tannin extracted into wine at different grape maturity levels were also investigated.

The extent of variation in Shiraz skin tannin distribution and cell wall structure and its tannin binding capacity was also investigated across a range of environmental conditions, including; Shiraz grapes grown with low, medium and high vigour canopies on Schwarzmann rootstock in Sunraysia, Australia; Shiraz grapes grown on Paulsen rootstock and own roots in Sunraysia, Australia and Shiraz grapes grown on Schwarzmann rootstock in the cooler growing region of Glenrowan, Australia.

Determination of the tannin distribution in grape seeds, skin and wine provided a more thorough characterisation of tannin than has previously been reported.

Grape seed tannin distribution was similar between varieties, whereas skin tannin distribution was influenced by varietal and environmental factors such as season and vine canopy vigour. The distribution of wine tannin was similar to grape skin with a DP less than 20. These results suggest that tannin above DP 20 are not extracted from grapes into wine during winemaking as they remain entrapped within the cell wall. A more thorough characterisation of the variation and structure of individual tannins below DP 20 would help to elucidate the tannins which are most important to wine quality.

The polysaccharide composition of grape skin and whole berries (seeds removed) varied considerably, with differences also observed between Shiraz and Cabernet Sauvignon grapes. However, there was no consistent trend in polysaccharide composition associated with maturity for either variety. There was also no link between polysaccharide composition and the tannin binding capacity of cell walls. Characterisation of polysaccharide composition and tannin binding capacity did not provide any indication of the amount of tannin that might be extracted into wine. However, the amount of cell wall material measured in grapes correlated with the amount of tannin extracted into wine. The amount of tannin extracted into wine is most likely influenced by cell wall structure such as the thickness or density of the skin cell wall rather than the composition of tannins and polysaccharides. However, the ratio of anthocyanin to tannin may also play a critical role in the stability of tannin during extraction and wine aging.

DECLARATION

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STATEMENT OF AUTHORSHIP

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CHAPTER 1. GENERAL INTRODUCTION

Condensed tannin is a member of a class of grape secondary metabolites known as flavonoids (*1*). Tannin plays an important role in plant defence as its astringent and bitter attributes act as deterrents to herbivores (*1*). Tannins are most commonly defined as phenolic compounds of molecular weights between 500 and 3,000 with the ability to precipitate alkaloids, gelatine and other proteins (*1-3*). This characteristic of tannin explains its astringent properties, which are caused by precipitation of proteins present in saliva (*4, 5*).

Tannin contributes to the organoleptic properties of wine, in particular astringency, wine colour and aging stability (*6-8*). The importance of tannin to wine quality is recognised by the Australian wine industry, but the influence of grape tannin structure and composition remains unclear. With limited understanding of how tannin influences wine quality, tannin management both in the vineyard and winery remains a challenge.

THE GRAPE BERRY

Wine is made by the fermentation of fruit harvested from the grapevine. Wine quality, determined by a combination of appearance, aroma, flavour and mouthfeel, is largely a reflection of the grape berry composition at harvest (*9*, *10*). Approximately 80 % of the grape berry is composed of water, but it is the sugars, organic acids, flavonoids and volatile compounds that make up the remainder of the grape berry that contribute to the colour, aroma and flavour characteristics of wine (*10, 11*).

Flavonoids in grape berries

Flavonoids are the largest class of plant polyphenols that contribute to wine quality (*1*). Plant polyphenols are secondary metabolites characterised by their water solubility, molecular weight, intermolecular complexation and structural characteristics (*1*). Flavonoids are based on a skeleton structure composed of a chroman ring bearing a second aromatic ring (Figure 1.1) (*1*). Flavonoids and the other classes of plant polyphenols, including glycosides, esters and hydroxycinnimates have been extensively reviewed (*1*).

Flavonoids found in grape berries include anthocyanins, flavonols and tannins. The biosynthesis of flavonoids comes from the successive modification of phenylalanine produced in the Shikimate pathway that ends with anthocyanin production (*12*). Flavonols and condensed tannins are the products of intermediates in the pathway (*12*).

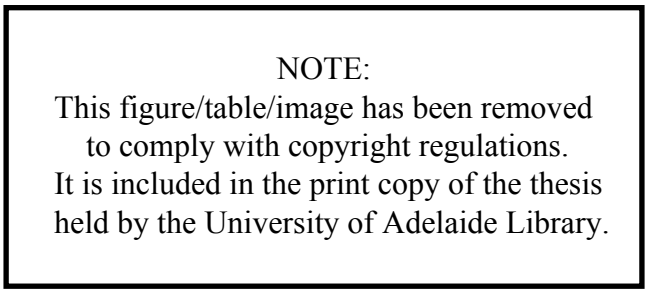

Figure 1.1. Generic skeleton structure of flavonoid compounds depicting a chroman ring C bearing a second aromatic ring B (*1*).

In the grape berry, tannins are located within the cell wall and vacuole of seeds and skin (*13-15*) and play a role in plant defence as herbivore deterrents and antifungal and antibacterials (*16*). Anthocyanins are located in the vacuole of skin cells and are responsible for the pigmentation and colour of red grapes. Anthocyanins play roles in UV protection, pollinator attraction and seed dispersal agent attraction (*17, 18*). The flavonols protect the plant from UV radiation damage and are located in the vacuole of grape skin (*12, 19, 20*).

As antioxidants, flavonoids are highly reactive, forming oligomers and polymers and complexes with other flavonoids, metal ions and numerous other molecules (*1, 21-23*).

Tannins in grape berries

In the grape berry, condensed tannins are polymers composed of flavan-3-ol subunits, typically linked via interflavan bonds between the C-4 and C-8 carbon atoms, and less commonly C-4 and C-6 atoms (Figure 1.2) (*1*).

Figure 1.2. Epicatechin dimers indicating the differences in C4-C8 and C4-C6 interflavan linkages and the position of functional groups (*1*).

The most common flavan-3-ols found in the grape berry are (+)-catechin, (-)-epicatechin, (-)-epicatechin-3-O-gallate and (-)-epigallocatechin (Figure 1.3). The multiple combinations of these four different subunits via two possible linkage positions and with varying polymer length gives rise to the many unique chemical structures, which makes tannin characterisation a complex and difficult task.

Current methods for tannin analysis include precipitation of tannin with protein such as bovine serum albumin (*24*) or methyl cellulose (*25*) to measure total tannin. Compositional

analysis of tannin requires acid-catalysed cleavage in the presence of a nucleophile such as phloroglucinol (*26*), which then allows tannin subunits to be separated and quantified using high performance liquid chromatography (HPLC).

A key shortcoming of existing tannin analysis methods for grape berries is the efficiency of extraction of tannin from skin and seed components. During berry development, the amount of tannin decreases and has been attributed to a decrease in tannin extractability. Tannin becomes more difficult to extract from the grape berry due to binding interactions with the grape cell wall (*13, 27-29*).

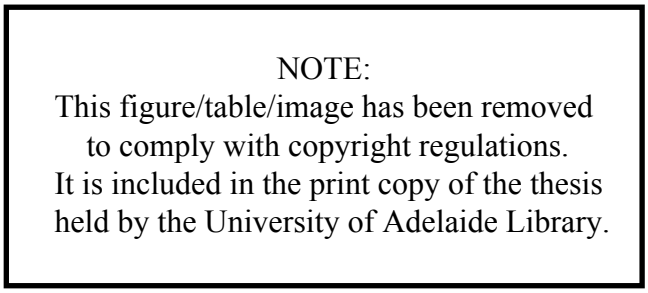

Figure 1.3. A generic grape tannin polymer depicting the terminal and extension subunits joined by interflavan bonds. R_1 and R_2 denote possible functional groups that differentiate the four possible subunits (Catechin, Epicatechin R_1=H, R_2=H; Epigallocatechin R_1=OH, R_2=H; Epicatechin gallate R_1=H, R_2=Gallic Acid) (*30*).

Grape berry cell walls

It is thought that tannin interactions with polysaccharides and structural proteins within the cell wall can influence extraction (*13, 29, 31, 32*). The cell wall structure of the grape berry is based on the type I model of primary plant cell walls (*33*). The cell wall is composed of three structural layers, a cellulose-xyloglucan framework, a pectin matrix and cross-linked structural proteins. The cellulose-xyloglucan framework comprises more than 50 % of the

total primary cell wall material. This is embedded in a pectin matrix, which accounts for 25 to 40 % of the cell wall material, which is locked into shape by cross-linked structural proteins that range in content from 1 to 10 % of the total composition.

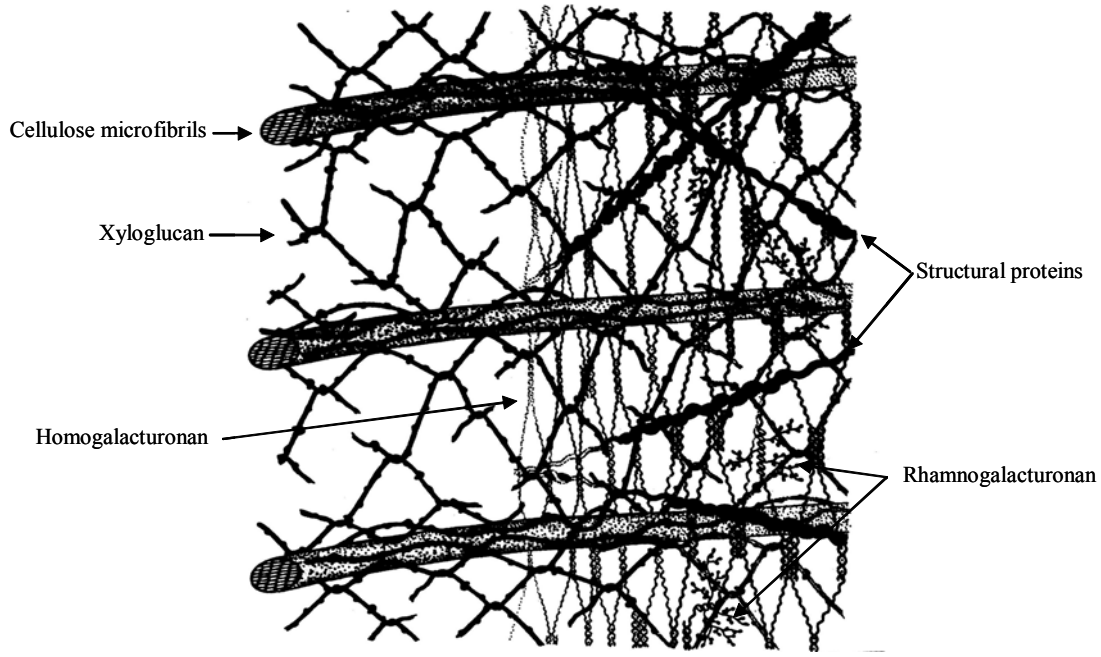

Figure 1.4. Plant cell wall structure depicting the cellulose-xyloglucan framework interlocked with pectic polysaccharides and structural proteins (*33*).

The cellulose and xyloglucan backbones are composed of β-(1,4) linked D-glucosyl sugar residues (*33, 34*). The xyloglucan backbone is branched with α-D-xylosyl residues, which undergo further branching by the addition of β-D-galactose, α-L-arabinose and α-L-fucosyl (*35-37*).

The main compositional pectic polysaccharides are homogalacturonan, rhamnogalacturonan I (RGI) and rhamnogalacturonan II (RGII) (*34*). Homogalacturonans consist of an α-(1,4)-D-galacturonosyl acid backbone and have a high degree of methyl esterification. The rhamnogalacturonans differ from homogalacturonan by the high number of branched side chains, which contain arabinosyl, galactosyl and arabinogalactosyl sugar residues. The backbones of the rhamnogalacturonans differ from each other with RGI

consisting of repeating units of α-(1,4)-D-galacturonosyl and α-(1,2)-L-rhamnogalacturonosyl acids and RGII composed of at least eight α-(1,4)-D-galacturonosyl acid units (*34*).

The structural proteins consist primarily of extensin, hydroxyproline and arabinogalactan (*33, 34*). The backbones of the structural proteins can be highly glycosylated with the polysaccharide portion of arabinogalactan-protein accounting for more than 90 % of the molecule (*34*).

Polysaccharide composition

While the skin accounts for only 5 to 12 % of the fresh weight of berries, 75 % of grape berry cell walls are located in the skin (*38, 39*). In the skin, the neutral polysaccharides such as cellulose, xyloglucan and arabinogalactan proteins account for between 30 and 40 % of the cell wall structure (*40*), while the pectic polysaccharides account for approximately 20 % of skin cell walls with 62 % methyl esterification (*40*). The cell walls of the grape berry flesh are composed mainly of cellulose and pectic polysaccharides (*38, 41, 42*). There is approximately two to three fold more xyloglucan in skin cell walls than the flesh, with xyloglucan accounting for approximately 2 % of all cell walls in the grape berry (*38, 39*). The composition of xyloglucan in the flesh and skin of grape berries have similar glycosyl residue composition. Glucose, xylose and galactose are the major sugars with smaller amounts of mannose, fucose and arabinose also present (*39, 40*).

Of the pectic polysaccharides, homogalacturonan accounts for 80 % of grape berry cell walls. There is three fold more rhamnogalacturonan I (RGI) than rhamnogalacturonan II (RGII), with RGII accounting for less than 5 % of the cell wall (*38, 40, 42*). However, the flesh contains two fold more pectin than the skin (*38*). Arabinogalactan proteins are also more abundant in grape berry tissue with the flesh containing two fold higher concentrations than skin tissue (*38, 43*).

Polysaccharide composition has been observed to vary in flesh tissue between grape varieties with differences observed in the amount of cellulose, xyloglucan and pectic polysaccharides (*42*). However, differences observed between cultivars may possibly reflect variations in berry maturity and the changes in polysaccharide composition associated with berry softening (*39*). Changes in pectic polysaccharides and a decrease in neutral sugars such as galactose are the most common changes reported during berry development (*37, 44-46*).

Differences in skin polysaccharide composition have also been observed between grape varieties, with differences in the amount of cell wall material, neutral sugars, uronic acids, and the degree of methylation and acetylation having been reported (*46*).

TANNIN IN WINE

Condensed tannins in the wine play a significant role in wine astringency, bitterness, colour stability and aging potential (*6-8, 47*). Astringency and bitterness are crucial to overall wine flavour providing a balance to other sensory characteristics such as fruit, aroma, flavour, acidity and sweetness (*9*). Bitterness is a taste mediated by sensory receptors on the tongue (*48, 49*), while astringency is best described as a 'dryness' in the palate and a 'pucker' like sensation experienced when tannins precipitate with proteins present in the saliva and polysaccharides that lubricate the mouth (*1, 8, 49, 50*).

Variation in tannin content, composition and size distribution are likely to determine mouthfeel and aging properties, however it is unclear how particular tannins or classes of tannins are related to different sensory and chemical characteristics of wine (*51*). The reactions of tannin in wine are a dynamic process with the structure and composition of tannin changing considerably as wine ages (*52*). The astringent perception of tannin in

wine is also modulated by interactions with other wine components such as ethanol, glycerol, salts, acids and macromolecules making it very difficult to characterise the specific contribution of tannins to the sensory properties of wine (*53*).

Polymer length

It has been well established that astringency increases and bitterness decreases with tannin polymer length (*48, 54-57*).

Although not considered as tannin, the monomeric flavan-3-ols have long been known to contribute to bitterness (*58*). As the polymer length increases from monomers to trimers, bitterness intensity and duration decreases, while astringency increases (*48*). In wine, astringency has been reported to increase with increasing polymer length to an average polymer length of 20 subunits (*47, 59*). It is thought that astringency continues to increase with polymer length as high molecular weight tannins are readily precipitated by protein (*60*).

Early research attributed a loss of astringency with wine ageing to tannin polymerisation and spontaneous precipitation (*59*). More recently, the decrease in astringency with aged wine is thought to be a result of structural changes to tannins such depolymerisation to form lower molecular weight material and polymeric pigments (*51, 60, 61*).

Composition and structure

The composition of subunits present in tannin and their positional linkage can influence tannin reactivity and affects mouthfeel. Compositional differences in tannin may determine tannin structure and the accessibility of interaction sites and molecular conformation related to astringent perception (*51*). There is relatively little known about how differences in tannin composition influence mouthfeel. These differences are likely to influence sub

quality parameters of astringency rather than overall astringency, which involves in depth descriptive sensory analysis.

The presence of the subunits epicatechin gallate and epigallocatechin are thought to influence astringency. An increase in the degree of galloylation by the constituent epicatechin gallate is reported to increase the coarse perception of tannin (*47*). In contrast, an increase in the presence of epigallocatechin decreases the perception of 'coarseness' (*47*).

The influence of galloylation on astringency is strong. Although polymer length is thought to increase astringency, short tannins with high galloylation are perceived similarly in overall astringency as larger tannins with low galloylation (*62*).

The presence of the monomeric flavan-3-ols, catechin and epicatechin subunits in the polymer, may also influence the overall astringency. Higher concentrations of catechin and epicatechin increase both bitterness and astringency (*56*) with epicatechin having a higher maximum intensity and longer persistence of bitterness and astringency than catechin (*63*).

The specific linkage of subunits also seems to influence astringency as a catechin-catechin dimer linked by a 4-6 bond is more bitter a catechin-catechin dimer linked by a 4-8 bond (*48*).

Associations with other compounds

During the winemaking and aging process, tannin reacts and forms complexes with other compounds to influence colour and mouthfeel (*64*). The wine conditions such as pH and the presence of other compounds such as anthocyanins, flavonols and polysaccharides will also influence the tannin structures that form in the final wine (*51*).

Copigmentation and acetylation

In young red wines, colour is primarily due to free or monomeric anthocyanins, but as wine ages anthocyanins combine with condensed tannins to form pigmented and colourless polymers (*51, 65, 66*).

Tannin and anthocyanins are both relatively unstable species that can undergo various types of chemical reactions (*67-69*).

Under the mild acidic conditions of red wine, tannin undergoes spontaneous cleavage of the interflavan bonds to create a reactive carbocation intermediate (*51, 70, 71*).

Tannin carbocations can react with anthocyanins to form colourless compounds, which can undergo further reactions to produce compounds ranging in colour from orange to red and violet (*7, 72*). It is thought that incorporation of anthocyanins into the tannin structure may decrease astringency (*51*).

Acetylation of the tannin carbocation may also occur. Under acidic conditions, aldehydes form a reactive species, which stabilises by forming a new carbocation with tannin, which then reacts with other tannin molecules or anthocyanins. Acetylation creates new polymer structures linking tannins and anthocyanins by ethyl cross-bonds (*51, 72*). Given that astringency increases with polymerisation, it is thought these reactions may enhance astringency (*51*). Studies on the sensory properties of tannins derived from these reactions are scarce as the isolation and characterisation of these complex structures is difficult. It is also not known what type or structure of tannin in finished wine will lead to more favourable quality characteristics as wine ages.

Polysaccharides in wine

Polysaccharides influence astringency and colour stability by reducing the capacity of tannin to bind with other compounds (*73-75*). The polysaccharides found in wine are grape derived rhamnogalacturonan II and arabinogalactan proteins as well as yeast derived

mannoproteins arising from the addition of yeast for fermentation during the winemaking process (*75*). Arabinogalactan proteins and mannoproteins are both considered polysaccharides due to the oligosaccharide chains which represent 90 % of the molecule (*75*).

Polysaccharides can influence tannin in various ways (*74*). Rhamnogalacturonan II (RGII), present as a dimer in wine, forms complexes with tannin by acting as a bridge between tannin molecules (*74*). This increases the size of tannin molecules, but may prevent copigmentation of tannin with anthocyanin, thereby influencing colour stability. These complexes also reduce the capacity of tannin binding with proteins present in the saliva, which therefore reduces astringency (*74, 75*).

While RGII is present in wine as a dimer, arabinogalactan-proteins and mannoproteins have much larger molecular weights. As such, arabinogalactan-proteins and mannoproteins form soluble complexes with tannin by absorbing the tannin molecule within their structures preventing tannin from reacting with other compounds (*74*).

TANNIN EXTRACTION

The reactions of tannin in wine begin as tannin is extracted from the grape seeds, skins and stems during maceration in the winemaking process (*10, 76*). Factors such as maceration time, fermentation temperature, enzyme activity and other winemaking additives, plus initial grape tannin composition can all play a role in determining the eventual tannin content of red wine and its influence on wine quality (*27, 32, 66*).

The mechanisms behind grape tannin extraction are not fully understood. However, it is thought that grape cell walls bind tannin reducing its extractability (*13, 27, 29, 77*). Further research is required to better understand the physio-chemical processes involved.

Winemaking process

The process of red winemaking involves the extraction of tannins and anthocyanins during fermentation with skin contact (*10*). Following crushing and destemming, the must is placed in a fermentation vessel and inoculated with yeast (*10*). The skins, which form a floating cap, are mixed with the fermenting juice at regular intervals to enhance extraction. This process, known as maceration, extracts the colour, tannin and flavour of red wine and varies from two or three days to serveral weeks depending on the desired style of wine (*9, 10*). Once the desired amounts of colour, tannin and flavour have been extracted, the fermentation vessel is drained, the remaining pomace is pressed and the skins and seeds are removed (*9, 10*). After pressing, the wine is fermented to 'dryness' (<2.0 g/L sugar) and then often undergoes malo-lactic fermentation (*10*). Hydrolysable tannins can be introduced to the wine either through addition of oak chips, planks or powder during fermentation or by aging the wine in oak barrels (*10*).

Interaction with cell walls

Poor extraction of tannin during the winemaking process is thought to result from tannin binding to the cell wall material of the grape berry (*13, 27-29, 31*). Once grape material is removed from the wine at pressing, tannin can no longer be extracted. Tannin can bind to polysaccharides in the cell wall through hydrogen bonding and hydrophobic interactions (*78, 79*). The formation of hydrogen bonds occurs between hydroxyl groups of tannins and the oxygen atoms within cross-linking ether bonds of sugars present in cell wall polysaccharides (*79, 80*). The strength of these interactions is enhanced by the gel-like structure of the cell wall that encapsulates tannin within hydrophobic pockets and cavities (*79, 81*). The extent of tannin extraction is also influenced by the molecular weight, degree of galloylation and stereochemistry of tannin polymers (*74, 79, 80, 82*). Longer polymers

with a high degree of galloylation increase the number of potential binding sites, thereby increasing the strength of tannin binding. The stereochemistry and structure of the tannin polymer may also influence the number of accessible sites at which binding may occur. In addition to tannin polymer structure, the cell wall composition may also influence the strength of these interactions. For example, tannin shows a higher affinity for certain polysaccharides, such as pectin (*78*). As polysaccharide composition varies between grape cultivars (*42, 46*), the extent of tannin extraction will likely vary between cultivars, as tannin will have a higher affinity for different polysaccharides.

Influence of winemaking

During maceration, tannin is more readily extracted from some grape varieties than others (*9*). Winemaking techniques can be used to influence the rate and amount of tannin extracted to reach the desired wine style and quality. Factors that influence tannin extraction include maceration time, cap management, temperature and levels of alcohol (*9, 10*).

Longer maceration times, more frequent cap mixing, the presence of ethanol in the fermentation media and higher fermentation temperatures enhance tannin extraction (*83, 84*). A longer maceration time will lead to a wine higher in tannin content as the skins and seeds are in contact with an ethanol rich media for a longer time (*84*).

Tannin extraction can also be enhanced by the addition of commercial enzymes such as pectinases, which have the ability to degrade cell wall polysaccharides to release tannin from the cell wall (*85, 86*). However, excessive tannin extraction can produce undesirable astringent characteristics that require fining to improve wine quality. Fining agents such as gelatine and other proteins can be added to wine to reduce astringency (*10*). Fining agents selectively precipitate high molecular weight tannins that are particularly astringent without significantly altering the wine composition (*51*).

Winemaking techniques aim to manage the extraction of tannin to achieve a balance between desirable mouthfeel characteristics and other quality parameters of wine, such as colour, sugar, alcohol, flavour and aroma, but there is a limit to what they can achieve.

CONCLUSIONS AND PROJECT AIMS

The Australian wine industry's ability to produce quality wine at competitive prices is a key factor influencing success in international wine markets. Australia faces increasing competition from New World wine producers such as Chile and South Africa who have the ability to export wine at a lower production cost than Australia. To ensure sustainability, the Australian wine industry must continue to improve the quality of wine it produces at all price points to remain competitive against other New World wine producers and maintain its share of global markets.

Harvest measurements of grape quality attributes such as colour, sugars and acids are generally indicative of the concentrations observed in the resulting wine. However, the level of tannin measured in the grape is rarely representative of that measured in wine. This may be due to tannins binding to polysaccharides in the cell wall, which prevents tannin extraction from grapes during winemaking. The strength of binding between tannins and polysaccharides is likely to be influenced by differences in both tannin and polysaccharide composition, which varies with variety, grape maturity and in response to environmental conditions such as climate and viticultural management practices.

The extent to which polysaccharide composition varies in wine grapes is unclear. Earlier studies investigating cell wall and polysaccharide composition in grape berries have focused on compositional changes that occur with fruit softening with few comparisons

that consider variety and viticultural management practices. It is also unclear whether differences in cell wall composition affect the capacity of cell walls to bind tannins.

Variation in tannin composition likely influences the strength of tannin interactions with cell walls. The type and amount of tannin extracted into wine is expected to depend on the polymer length distribution and composition of tannin. However, previous studies have focused on average polymer length and composition rather than actual distribution. Yet it is likely that the distribution of tannin polymer length will also vary across varieties and according to viticultural management practices.

A more comprehensive study is required to explore the extent of variation in both tannin and polysaccharide composition in the grape berry in order to better understand how grape composition influences the type and amount of tannin extracted into wine and the resultant impact on mouthfeel. The knowledge gained from this understanding will enable vineyard and winery management practices to be tailored to optimise tannin extraction, thereby improving wine quality.

This project describes an investigation into the tannin and polysaccharide composition of wine grapes, the relationship between tannin composition, polymer length, polysaccharide composition, the tannin binding capacity of grape cell walls and the amount and type of tannin extracted into wine. It also investigated the influence of several environmental factors, such as climate and vineyard variability that may determine variation in tannin and cell wall composition.

Specifically, the aims of this project were:

- To characterise tannin distribution in Shiraz and Cabernet Sauvignon wine grapes
- To characterise the polysaccharide composition of Shiraz and Cabernet Sauvignon grape berries and the tannin binding capacity of grape cell walls

- To determine environmental factors that influence variation in tannin and polysaccharide composition and the tannin binding capacity of grape cell walls

CHAPTER 2. CONDENSED TANNIN DISTRIBUTION IN THE SKIN, SEED AND WINE OF SHIRAZ AND CABERNET SAUVIGNON WINE GRAPES

INTRODUCTION

Condensed tannins derived from the grape berry play a significant role in wine astringency, bitterness, colour stability and aging potential. Variation in tannin content, composition and polymer length are likely to determine mouthfeel and aging properties of wine. The content and composition of tannin can vary according to grape cultivar, region and vineyard management treatments (*87-89*). The measurement of tannin in the grape berry at harvest is typically not representative of tannin extracted into wine (*90*). It is thought that the extraction of tannin from grapes is affected by interactions between tannin and cell wall material in the grape (*13, 27, 28, 29, 31, 32*). Such interactions may be influenced by the nature of tannins, with the extent and strength of the interactions between tannins and cell walls depending on the polymer length, degree of galloylation and stereochemistry of the tannin molecule (*74, 78-80*).

The average subunit composition and degree of polymerisation (DP) of grape berry tannins have been investigated in a number of different grape varieties and at different stages of berry development (*14, 15, 26, 29, 77, 89, 91, 92*). However there are only a few studies that have examined the distribution of polymer length in grape berries (*91, 93-95*).

The following paper provides a thorough characterisation of the distribution of tannin in the skin, seeds and wine derived from Shiraz and Cabernet Sauvignon grapes, based on determination of polymer length, concentration and subunit composition.

Hanlin, R.L., Kelm, M.A., Wilkinson, K.L. & Downey, M.O. (2011). Detailed characterization of proanthocyanidins in skin, seeds, and wine of Shiraz and Cabernet Sauvignon wine grapes (*Vitis vinifera*).
Journal of Agricultural and Food Chemistry, v. 59 (24), pp. 13265 -13276

CHAPTER 3: EXTRACTION OF CONDENSED TANNINS FROM SHIRAZ AND CABERNET SAUVIGNON GRAPES INTO WINE

INTRODUCTION

Extraction of condensed tannins during fermentation

The extraction of condensed tannins during fermentation is a complex process, which makes it difficult to predict potential wine tannin content based on grape berry measurements. Only a small proportion of tannin measured in grapes is extracted during winemaking, the majority of tannins remain in the grape matrix and are removed at pressing (*32*). Adding to the complexity of tannin extraction, is the localisation of tannin within the skin and seed of the berry and the different extraction rates of tannin from these tissues. The different extraction rates are most likely influenced by differences in tannin structure between skin and seed derived tannins, as well as the structural properties of these different grape tissues.

Seed tannins contain similar proportions of the polymer subunits catechin, epicatechin and epicatechin gallate, whereas skin tannin is primarily composed of epicatechin and epigallocatechin, with small amounts of epicatechin gallate and catechin (*91, 96*). In addition to tannin structure, seed tannin extraction is thought to be influenced by the 'leakiness' of seed parenchyma cells and the thickness of the seed's outer cuticle, which can prevent tannin diffusion (*97*). Skin tannin extraction may also be influenced by how strongly tannin is bound to the cell wall matrix preventing its extraction (*98*). Differences in structure and composition of grape berry polysaccharides may significantly affect how strongly tannin remains bound to the

cell wall matrix. Additionally, once tannin is extracted from the skin and seed, there are additional binding opportunities within the fermentation matrix.

While tannin extraction is likely to vary depending on grape composition, it is generally observed that tannin concentration increases with skin and seed contact until pressing (*99-101*). Tannin extraction has been studied in relation to many variables including temperature, alcohol content, skin and seed contact time amongst others, many of which can increase potential phenolic extraction (*102-107*).

Skin and seed tannin extraction have also been investigated separately both during fermentation and in model wines to investigate the transfer of skin and seed tannin composition into wine (*83, 108-111*). To date, there have been no studies that compare grape characteristics that might influence the rate of tannin extraction. The majority of studies have investigated the influence of different winemaking variables and individual grape tissue characteristics using a single grape variety.

It has been hypothesised that tannin extraction is also influenced by grape maturity since tannin and cell wall composition are likely to change during grape berry development (*98*). Differences in tannin and cell wall composition are likely to influence how strongly tannin remains bound to the cell wall matrix, thereby impeding its extraction during fermentation. Tannin and cell wall composition also differ between varieties and in response to various environmental influences (*42, 45, 46, 87*). Tannin extraction during fermentation has not been investigated with respect to how differences in grape composition such as maturity, tannin and cell wall composition might influence the type, rate and amount of tannin extracted.

An aim of this chapter was to employ micro-scale fermentation to investigate the effect of maturity on extraction rate, content and composition of condensed tannins in

two wine grape varieties, Shiraz and Cabernet Sauvignon grown in Sunraysia, northwest Victoria.

The use of 100-berry micro-fermentations was adopted as a research tool to enable the rapid assessment of multiple wine parameters under controlled, replicated conditions. Individual ferments represent a replicate that was destructively analysed at each sampling point. This eliminates potential treatment effects associated with repeated sampling of larger ferments and overcomes the challenge of taking representative samples comprising must, cap and lees from a larger ferment.

Perception of astringency in red wine

Astringency is a multi-perceptual phenomenon (*8, 112*). While astringency has been broadly described as a 'drying', 'roughing' and 'puckering' sensation, more than 20 different descriptive astringent terms have been used to characterise astringency in red wine; for example, 'silk', 'emery paper', 'course', 'smooth' and 'chalky' (*8*).

The descriptive characteristics of astringency are elicited by both physical and chemical properties involved in the mechanism of astringency. Chemically, the perception of astringency results from the binding and subsequent precipitation of tannins with salivary proteins and glycoproteins that lubricate the oral cavity (*4, 5*). These interactions and the resulting perception of astringency are influenced not only by the concentration and structure of tannins, but also properties of wine such as pH, acidity, ethanol concentration, sweetness and viscosity (*113-117*). Other compounds present in wine such as polysaccharides, peptides, ions, volatile compounds, and low molecular weight phenolics such as hydroxycinnamates, coumaric acid and caffeic acid may also contribute to perceived astringency (*64*).

Tannin concentration is the primary contributing factor to astringent perception, with increasing tannin concentration increasing the overall intensity of astringency (*56,*

118). Structural features of tannin such as increased polymer length and galloylation also increase the overall intensity of astringency (*47*). Galloylation has been associated with specific astringent descriptors such as the level of 'coarseness', while epigallocatechin has been correlated with the astringent perception of 'smoothness' (*47*). Wine chemical properties such as an increase in ethanol, pH, sweetness and viscosity can also decrease astringent perception (*113, 114, 117*).

The reduced astringency observed during wine aging has been attributed to the cleavage of tannins to form smaller polymers, the formation of colloids with polysaccharides and interactions between tannins and anthocyanins to form pigmented polymers (*116*). In the mildly acidic conditions of wine, the interflavan bonds within tannins are cleaved to form smaller, less astringent polymers (*116*). Tannin polymers may also form colloids with polysaccharides such as rhamnogalacturonan II in wine, which has been shown to decrease tannin astringency (*116*). The formation of pigmented polymers during wine aging may contribute to a decrease in astringency as it is thought that pigmented polymers in wine do not contribute to astringency (*49*).

The influence of different structural and chemical properties of tannin on the descriptive astringent terms has not been thoroughly characterised; neither has the effect of grape composition on the astringent properties of red wine been determined.

The second aim of this chapter was therefore to determine the astringent properties of Shiraz and Cabernet Sauvignon wines that have previously been characterised for grape and wine tannin composition. To reduce the influence of variables such as sugar and ethanol on astringency, wine was made from grapes harvested at the same level of sugar ripeness.

MATERIALS AND METHODS

Sample collection

Shiraz and Cabernet Sauvignon grape berries were harvested in 2009 from a single vineyard located in the Sunraysia region of southeast Australia (34°27'S,142°14'E). Grape bunches were harvested at three maturity levels being: 19.7, 22.3 and 23.4 °Brix for Shiraz and 19.4, 20.1 and 23.8 °Brix for Cabernet Sauvignon. Approximately 5 kg of whole bunches were collected from 10 panels and stored at -20°C until micro-fermentation.

An extra 75 kg of Shiraz and Cabernet Sauvignon fruit was harvested at 23.4 and 23.8 °Brix respectively for the small scale winemaking described in Chapter 2. These wines were used for descriptive sensory analysis.

100-Berry micro-ferments

Grape berries were removed from bunches while still frozen and allowed to defrost overnight. Random samples of 100 berries (in triplicate) were collected to enable pH, titratable acidity, skin weight and berry weight to be determined. For each harvest date and variety, 100 berries were counted into each of 30 plastic polypropylene fermentation containers (300 mL). Sulphur dioxide (50 ppm) was added to each container as potassium metabisulphite and samples were allowed to reach fermentation temperature overnight (18°C). Prior to crushing, additional sulphur dioxide (50 ppm) was added to each container. Each sample was placed into a resealable bag and crushed by pressing all grape berries flat by hand. The crushed grape berries were returned to the fermentation container and pH adjusted to 3.4 by addition of tartaric acid. Diammonium phosphate (150 ppm) was added to each container and gently mixed by rolling the container. Re-hydrated yeast (0.2 g/L,

Lalvin EC1118) was added to each ferment at a rate of 2 mL/L of juice. The container lid was loosely replaced and fermentation commenced overnight. Three containers were removed daily for each variety and harvest date and analysed daily for total soluble solids (Anton Paar density meter, Graz, Austria) and phenolic content and composition by high performance liquid chromatography (HPLC). The remaining ferments were plunged twice daily using a small potato masher. After seven days the remaining micro-ferments were pressed using a citrus squeezer lined with cheese cloth and the must collected in a 150 mL plastic polypropylene container. Micro-fermentations continued for three more days until all samples had been collected for analysis (ie. 10 days in total).

Tannin analysis

For must and wine analysis, ethanol was first removed from a 2.5 mL aliquot under reduced pressure (35^{o}C). The evaporated sample was centrifuged (5 minutes, 16,100 x g) and the supernatant applied to a C_{18}-SPE cartridge (300 mg, Alltech, Grace Davison Discovery Sciences, Australia) previously activated by methanol (100 %, 5 mL) and water (5 mL, Milli-Q, Millipore, Billerica, USA). The remaining precipitate was washed by resuspending in water (1 mL), centrifuging (5 minutes, 16,100 x g) and the supernatant applied to the SPE cartridge. The SPE cartridge was then washed with water (9 mL) to remove monomeric material, anthocyanins, sugars and organic acids. The remaining sample was eluted with methanol (100 %, 9 mL) and an aliquot (1 mL) of the sample was dried under reduced pressure prior to HPLC analysis.

Tannin concentration, average polymer length and subunit composition of must and wine were determined according to the method described by Hanlin et al. (*89*) using an 1100 Agilent HPLC (Palo Alto, USA). Grape skin and seeds were also analysed for tannin content and composition prior to fermentation using the same method.

Wine colour analysis

Red wine colour measurements were performed with a micro-plate spectrophotometer (SpectraMax Plus384 Absorbance Microplate reader, Molecular Devices, Sunnyvale, USA) using polystyrene flat bottom 96 well plates (Greiner Bio-One, Frickenhausen, Germany). Red wine colour parameters included wine colour density, wine hue, total anthocyanins, ionised anthocyanins, total red pigments and total phenolics and were determined using the methods developed by Somers and Evans (*119*) and Iland et al. (*120*).

Wine sensory analysis

Descriptive analysis of the both Shiraz and Cabernet Sauvignon wines was performed 12 months post fermentation, to quantitatively characterise organoleptic differences between the two varieties and amongst fermentation replicates. Wines were evaluated by a trained panel comprising staff and students from Adelaide University, 3 females and 6 males. Formal sensory analysis was conducted in isolated booths at 22-23^{o}C under neutral light conditions. Wines were presented as 30 mL samples in three digit-coded, covered, ISO standard glasses. Panelists rated the wines against 17 descriptive terms including two for appearance, 8 for

Table 3.1. Descriptive terms used for characterising the sensory properties of Shiraz and Cabernet Sauvignon wines.

Group	Descriptor	Abbreviation
Appearance	Colour	C
Appearance	Colour Intensity	CI
Aroma	Intensity	I
Aroma	Light Fruit	LF
Aroma	Dark Fruit	DF
Aroma	White Pepper	WP
Aroma	Black Pepper	BP
Aroma	Confectionary	Con
Aroma	Herbaceous	He
Aroma	Fruit	Fr
Flavour	Astringency	Ast
Flavour	Tannin Structure	TS
Flavour	Fruit	Fr1
Flavour	Body	Bo
Flavour	Acid	A
Flavour	Spice	Spice
Flavour	Length	L

aroma and 7 for flavour (Table 3.1).

The descriptive terms for each wine were rated using a 10 cm line scale with anchor points at each end of the scale marked 0 and 10. Distilled water was provided as a palate cleanser and panellists were forced to rest for 30 s between each sample.

Statistical analysis

The rate of micro-fermentation for average polymer length and tannin concentration was analysed by repeated measures analysis of variance (ANOVA) using XLSTAT Microsoft Excel software.

Sensory data was collected using the Fizz software (Version 1.3, Biosystèmes, Couternon, France). Mean ratings and Fischer's least significant differences for each attribute were calculated by ANOVA using the Fizz software. Differences among attributes for each variety were assessed with mixed model ANOVAS with judges considered a random effect. Principal component analysis (PCA) was also performed using the Fizz software application to show possible correlations between sensory and chemical data.

RESULTS

Micro-ferments

Prior to micro-fermentation, the concentration and subunit composition of skin and seed tannins were determined. Tannin compositions were similar to previous studies with seed tannin containing catechin, epicatechin and epicatechin gallate subunits and skin also containing epigallocatechin (Table 3.2) (*28, 29, 89, 121*). Seed tannin was composed of terminal subunits catechin, epicatechin and epicatechin gallate, all

present at around 30 to 35 % (Table 3.2). Seed tannin extension subunits were composed primarily of epicatechin representing around 70 %, followed by epicatechin gallate at around 24 % and catechin at 5 %. The proportion of epicatechin gallate in seed tannin was slightly higher in Shiraz than Cabernet Sauvignon for both terminal and extension subunits, but only by around 3 to 5 %.

Skin tannin was composed of terminal subunits catechin and epicatechin, with catechin representing around 69 to 77 % and epicatechin between 22 and 31 % (Table 3.2). Skin tannin extension subunits were primarily composed of epicatechin and epigallocatechin. In Shiraz, epicatechin and epigallocatechin were present at similar

Table 3.2. Composition of Shiraz and Cabernet Sauvignon grapes harvested at three maturity levels*.

	Shiraz			Cabernet Sauvignon		
Harvest date	12-Feb-09	23-Feb-09	04-Mar-09	23-Feb-09	04-Mar-09	26-Mar-09
Total soluble solids (ºBrix)	19.7 ± 0.1	22.3 ± 0.01	23.4 ± 0.03	19.4 ± 0.03	20.1 ± 0.01	23.8 ± 0.1
pH	3.95 ± 0.01	4.08 ± 0.01	4.08 ± 0.01	3.87 ± 0.02	3.93 ± 0.03	4.08 ± 0.04
Titratable acidity (g/L)	2.91 ± 0.03	2.88 ± 0.02	2.51 ± 0.02	3.75 ± 0.22	3.32 ± 0.3	2.97 ± 0.2
Berry weight (g)	1.07 ± 0.1	1.2 ± 0.1	1.23 ± 0.04	0.94 ± 0.1	0.87 ± 0.02	0.97 ± 0.02
Total skin tannin (mg/g skin)	3.4 ± 0.2	4.4 ± 0.1	5.6 ± 0.2	4.4 ± 0.8	3.3 ± 0.4	4.1 ± 0.3
Total seed tannin (mg/g seed)	27.5 ± 0.3	24.2 ± 0.9	21.6 ± 0.4	28.9 ± 0.9	26.4 ± 0.7	26.3 ± 0.1
Skin average polymer length	43 ± 0.7	43 ± 0.4	52 ± 0.3	48 ± 3.5	43 ± 2.1	48 ± 1.4
Seed average polymer length	6 ± 0.1	6 ± 0.2	6 ± 0.1	6 ± 0.1	6 ± 0.1	6 ± 0.1
% Skin tannin composition as extension and terminal subunits						
Epigallocatechin extension	45.3 ± 0.1	44.3 ± 0.1	44.8 ± 0.2	56.5 ± 0.2	53.6 ± 0.1	54.5 ± 0.1
Catechin extension	3.3 ± 0.03	3.2 ± 0.01	3.4 ± 0.03	2.3 ± 0.1	2.3 ± 0.02	2.3 ± 0.01
Epicatechin extension	45.3 ± 0.1	45.5 ± 0.1	44.6 ± 0.04	38.3 ± 0.2	41.2 ± 0.03	40.3 ± 0.1
Epicatechin gallate extension	6.1 ± 0.1	7.1 ± 0.02	7.3 ± 0.2	2.9 ± 0.1	2.8 ± 0.03	2.9 ± 0.03
Catechin terminal	72.5 ± 0.3	77.2 ± 0.3	77.4 ± 0.1	70.9 ± 1.1	68.5 ± 1.3	69 ± 0.4
Epicatechin terminal	27.5 ± 0.3	22.8 ± 0.3	22.6 ± 0.1	29.1 ± 1.1	31.5 ± 1.3	31 ± 0.4
Epicatechin gallate terminal	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
% Seed tannin composition as extension and terminal subunits						
Epigallocatechin extension	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Catechin extension	4.9 ± 0.04	4.4 ± 0.1	3.9 ± 0.1	6.3 ± 0.1	5.5 ± 0.03	5.4 ± 0.1
Epicatechin extension	68.7 ± 0.1	68.8 ± 0.1	69.8 ± 0.1	70.4 ± 0.1	71.4 ± 0.1	71.1 ± 0.1
Epicatechin gallate extension	26.4 ± 0.1	26.8 ± 0.1	26.3 ± 0.2	23.4 ± 0.04	23.1 ± 0.1	23.5 ± 0.1
Catechin terminal	28.9 ± 0.1	29 ± 0.4	28.4 ± 0.2	32.8 ± 0.1	38.1 ± 0.1	35.6 ± 0.1
Epicatechin terminal	36.6 ± 0.04	36.5 ± 0.4	36.7 ± 0.2	35.3 ± 0.1	32.7 ± 0.1	33.7 ± 0.1
Epicatechin gallate terminal	34.5± 0.1	34.5 ± 0.3	34.9 ± 0.3	31.9 ± 0.2	29.2 ± 0.1	30.7 ± 0.1

*Values are means of three replicates ± standard error n.d. = not detected

levels representing around 45 % each of extension subunits, while for Cabernet Sauvignon, epigallocatechin represented around 55 % and epicatechin represented around 40 %. Epicatechin gallate was also present in low levels as an extension subunit of both Shiraz and Cabernet Sauvignon skin tannin, but was around two-fold higher in Shiraz than Cabernet Sauvignon representing around 7 % of extension subunits in Shiraz.

The average polymer length of seed tannin was six subunits, while the average polymer length of skin tannin ranged between 43 and 52 subunits. During fermentation, the average polymer length of extracted tannin ranged between five and eleven subunits (Figure 3.1a). The tannin concentration of Shiraz increased gradually over the ten days of fermentation for all maturity levels (Figure 3.1b). In contrast, Cabernet Sauvignon tannin concentration increased rapidly during fermentation reaching a maximum concentration at pressing (Day 7) for all maturity levels, followed by a decrease to levels that were similar to Shiraz. After ten days, the tannin concentration

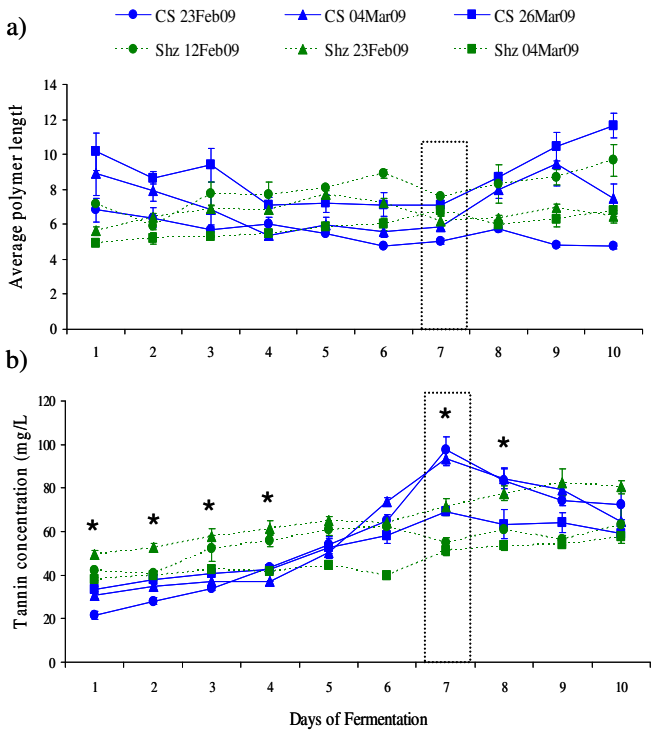

Figure 3.1. Rate of extraction during micro-fermentation for a) average polymer length and b) total tannin concentration (n=3). *Significant difference $p<0.005$ where there is a significant interaction for variety. Pressing is indicated by a box at day 7 of fermentation. CS = Cabernet Sauvignon, Shz = Shiraz.

ranged between 40 and 70 mg/L for Shiraz with the highest tannin concentration occurring in Shiraz harvested at 22.3 ^{o}Brix. Shiraz harvested at 19.7 and 23.4 ^{o}Brix had similar levels. For Cabernet Sauvignon, the maximum tannin concentration occurred at pressing (Day 7) being 95 mg/L for fruit harvested at 19.4 and 20.1 ^{o}Brix and 60 mg/L for fruit harvested at 23.8 ^{o}Brix. After pressing, the concentration of tannin decreased for all Cabernet Sauvignon samples ranging between 40 and 70 mg/L.

Table 3.3. Tannin composition as extension and terminal subunits of Shiraz throughout micro-fermentation*.

Days of fermentation	Epigallocatechin extension	Epicatechin extension	Epicatechin gallate extension	Catechin terminal	Epicatechin terminal
			Shiraz harvested 19.7 ^{o}Brix		
1	24.4 ± 0.1	64.9 ± 0.6	10.7 ± 0.5	85.5 ± 0.6	14.5 ± 0.6
2	23.3 ± 0.1	63.7 ± 0.1	12.9 ± 0.2	77.8 ± 0.03	22.2 ± 0.03
3	25.4 ± 0.3	60.8 ± 0.1	13.8 ± 0.3	71.8 ± 0.1	28.2 ± 0.1
4	23.8 ± 0.4	62.2 ± 0.6	14.1 ± 0.3	65.6 ± 1.2	34.4 ± 1.2
5	22.9 ± 0.6	63.0 ± 0.2	14.1 ± 0.8	64.7 ± 1.1	35.3 ± 1.1
6	22.0 ± 0.04	62.0 ± 1.0	16.0 ± 0.9	62.1 ± 0.4	37.9 ± 0.4
7	18.5 ± 0.5	66.0 ± 1.3	15.5 ± 0.7	55.9 ± 0.03	44.1 ± 0.03
8	20.7 ± 0.3	63.7 ± 0.6	15.6 ± 0.9	55.7 ± 1.0	44.3 ± 1.0
9	18.1 ± 0.3	68.1 ± 0.04	13.7 ± 0.3	48.1 ± 1.1	51.9 ± 1.1
10	18.3 ± 0.6	69.0 ± 0.1	12.7 ± 0.5	43.5 ± 1.1	56.5 ± 1.1
			Shiraz harvested at 22.3 ^{o}Brix		
1	29.4 ± 0.3	59.4 ± 0.6	11.2 ± 0.8	81.2 ± 0.4	18.8 ± 0.4
2	27.7 ± 0.2	56.6 ± 0.3	15.7 ± 0.5	73.2 ± 0.7	26.8 ± 0.7
3	26.1 ± 0.3	57.4 ± 0.2	16.7 ± 0.5	71.1 ± 1.1	28.9 ± 1.1
4	25.1 ± 0.2	55.7 ± 0.6	19.2 ± 0.8	70.3 ± 1.0	29.7 ± 1.0
5	25.8 ± 0.4	56.1 ± 0.2	18.1 ± 0.6	63.5 ± 0.3	36.5 ± 0.3
6	24.6 ± 0.3	56.7 ± 0.7	18.7 ± 0.6	63.8 ± 0.8	36.2 ± 0.8
7	22.8 ± 0.2	58.5 ± 0.3	18.7 ± 0.3	65.7 ± 1.1	34.3 ± 1.1
8	22.5 ± 0.1	59.7 ± 0.3	17.8 ± 0.3	65.7 ± 2.3	34.3 ± 2.3
9	22.7 ± 0.3	60.5 ± 0.1	16.8 ± 0.4	65.2 ± 2.6	34.8 ± 2.6
10	21.9 ± 0.3	62.8 ± 0.9	15.4 ± 0.7	64.0 ± 1.0	36.0 ± 1.0
			Shiraz harvested at 24.3 ^{o}Brix		
1	28.7 ± 0.5	58.6 ± 0.7	12.8 ± 1.3	81.1 ± 0.5	18.9 ± 0.5
2	28.9 ± 0.7	54.2 ± 1.1	16.9 ± 0.4	73.6 ± 1.1	26.4 ± 1.1
3	25.3 ± 0.4	56.2 ± 0.6	18.5 ± 0.9	69.3 ± 1.8	30.7 ± 1.8
4	25.3 ± 0.1	55.4 ± 0.4	19.3 ± 0.4	68.7 ± 0.8	31.3 ± 0.8
5	24.8 ± 0.1	56.3 ± 0.1	18.9 ± 0.04	66.4 ± 1.4	33.6 ± 1.4
6	23.8 ± 0.6	56.1 ± 0.5	20.0 ± 1.1	61.8 ± 1.8	38.2 ± 1.8
7	22.1 ± 0.5	59.8 ± 1.3	18.1 ± 0.9	62.1 ± 0.2	37.9 ± 0.2
8	21.2 ± 0.1	58.6 ± 0.3	20.1 ± 0.3	65.4 ± 1.9	34.6 ± 1.9
9	21.5 ± 1.1	60.2 ± 0.3	18.3 ± 1.3	64.6 ± 3.0	35.4 ± 3.0
10	19.9 ± 1.1	62.2 ± 1.4	17.9 ± 0.4	62.3 ± 0.9	37.7 ± 0.9

*Values are means of three replicates ± standard error

During fermentation, the tannin subunit composition of Shiraz and Cabernet Sauvignon were similar in all samples with the proportion of epicatechin gradually increasing throughout fermentation for both extension and terminal subunits (Table 3.3 and 3.4). The extension subunit epigallocatechin and terminal subunit catechin decreased in proportion in both varieties, while the extension subunit catechin was not detected. The extension subunit epicatechin gallate increased slightly during fermentation of Shiraz, but decreased in Cabernet Sauvignon.

Table 3.4. Tannin composition as extension and terminal subunits of Cabernet Sauvignon throughout micro-fermentation*.

Days of fermentation	Epigallocatechin extension	Epicatechin extension	Epicatechin gallate extension	Catechin terminal	Epicatechin terminal
Cabernet Sauvignon harvested at 19.4 °Brix					
1	24.5 ± 5.1	52.1 ± 5.0	23.4 ± 1.4	81.0 ± 10	19.0 ± 10
2	32.7 ± 0.5	48.1 ± 0.3	19.2 ± 0.8	64.3 ± 7.9	35.7 ± 7.9
3	30.2 ± 0.8	52.5 ± 1.8	17.2 ± 1.5	53.3 ± 2.1	46.7 ± 2.1
4	26.9 ± 1.0	57.8 ± 0.4	15.3 ± 0.7	50.2 ± 1.5	49.8 ± 1.5
5	24.5 ± 0.9	64.5 ± 0.8	10.9 ± 0.7	46.7 ± 2.0	53.3 ± 2.0
6	20.3 ± 0.7	70.0 ± 0.9	9.6 ± 0.3	44.9 ± 1.3	55.1 ± 1.3
7	15.8 ± 0.5	76.7 ± 0.4	7.5 ± 0.5	47.0 ± 0.4	53.0 ± 0.4
8	15.9 ± 0.7	77.5 ± 0.5	6.6 ± 0.3	44.6 ± 2.4	55.4 ± 2.4
9	19.8 ± 0.9	68.9 ± 0.5	11.3 ± 0.4	45.2 ± 1.3	54.8 ± 1.3
10	19.6 ± 0.1	71.3 ± 0.6	9.1 ± 0.6	43.0 ± 0.5	57.0 ± 0.5
Cabernet Sauvignon harvested at 20.1 °Brix					
1	25.5 ± 1.3	55.3 ± 0.5	19.2 ± 1.2	75.9 ± 6.0	24.1 ± 6.0
2	23.8 ± 1.7	59.7 ± 0.8	16.4 ± 1.0	65.7 ± 7.1	34.3 ± 7.1
3	24.8 ± 4.4	60.0 ± 4.5	15.3 ± 0.2	54.4 ± 1.8	45.6 ± 1.8
4	29.2 ± 0.5	55.6 ± 0.2	15.2 ± 0.3	55.7 ± 1.8	44.3 ± 1.8
5	23.2 ± 0.2	65.3 ± 1.3	11.5 ± 1.2	49.2 ± 1.1	50.8 ± 1.1
6	18.5 ± 1.0	72.2 ± 0.9	9.3 ± 0.2	46.6 ± 1.2	53.4 ± 1.2
7	13.7 ± 0.1	79.1 ± 0.02	7.1 ± 0.1	48.9 ± 1.2	51.1 ± 1.2
8	14.1 ± 1.9	79.5 ± 2.1	6.4 ± 0.2	49.0 ± 2.6	51.0 ± 2.6
9	15.4 ± 3.1	77.1 ± 2.3	7.5 ± 0.8	46.2 ± 2.6	53.8 ± 2.6
10	20.0 ± 1.5	69.7 ± 1.5	10.4 ± 0.4	49.4 ± 1.2	50.6 ± 1.2
Cabernet Sauvignon harvested at 23.8 °Brix					
1	25.6 ± 1.7	52.5 ± 0.6	21.9 ± 1.8	76.2 ± 6.0	23.8 ± 6.0
2	25.9 ± 1.9	56.5 ± 0.6	17.6 ± 1.5	61.7 ± 0.6	38.3 ± 0.6
3	24.6 ± 0.7	59.0 ± 3.0	16.4 ± 2.5	59.4 ± 0.9	40.6 ± 0.9
4	29.4 ± 1.2	52.1 ± 1.9	18.5 ± 1.1	58.5 ± 2.0	41.5 ± 2.0
5	26.3 ± 1.8	59.4 ± 1.8	14.4 ± 0.5	55.5 ± 1.2	44.5 ± 1.2
6	24.8 ± 1.3	64.2 ± 1.7	11.0 ± 0.4	52.0 ± 1.4	48.0 ± 1.4
7	21.9 ± 1.1	68.2 ± 1.0	9.9 ± 0.2	49.7 ± 1.4	50.3 ± 1.4
8	19.5 ± 1.7	72.1 ± 2.1	8.4 ± 0.5	52.5 ± 0.6	47.5 ± 0.6
9	16.2 ± 1.7	75.7 ± 2.1	8.2 ± 1.1	54.8 ± 1.9	45.2 ± 1.9
10	19.6 ± 1.1	71.1 ± 2.2	9.3 ± 1.2	56.1 ± 0.8	43.9 ± 0.8

*Values are means of three replicates ± standard error

At the end of fermentation, the extension subunit epigallocatechin represented around 19 % of extension subunits in both Shiraz and Cabernet Sauvignon, while the epicatechin extension subunit represented between 62 and 71 %. In Shiraz, extension subunit epicatechin gallate represented between 13 and 18 % of extension subunits, but was lower at around 10 % for all Cabernet Sauvignon samples. For terminal subunits, the proportion of catechin and epicatechin present at the start of fermentation was 75 to 85 % and 14 to 23 %, respectively. By the end of fermentation, the proportion of terminal subunits had changed, with catechin representing between 43 and 62 % and epicatechin increasing to between 37 and 56 %.

Chemical analysis of small scale wines

The tannin concentration and composition of small scale wines were determined at pressing, at the end of fermentation and after 12 months of aging (Table 3.5).

For Shiraz, the concentration of tannin increased from 92 mg/L at pressing to 117 mg/L at the end of fermentation. In comparison, the concentration of tannin in Cabernet Sauvignon decreased from 193 mg/L at pressing to 171 mg/L at the end of fermentation. During fermentation, Cabernet Sauvignon had a higher tannin concentration than Shiraz, but after 12 months of aging, the tannin concentration was similar for both varieties being around 76 mg/L.

The average polymer length decreased for both varieties between pressing and the end of fermentation and decreased further with aging. Both varieties had an average polymer length of around 10 subunits at pressing and around 5 subunits after 12 months of aging.

Tannin composition of the small scale wines was relatively similar for both varieties. Epigallocatechin represented around 30 % of the extension subunit composition at all

time points, while the proportion of epicatechin increased slightly after 12 months of aging, from 55 to 65 % of all extension subunits. The proportion of the extension subunit epicatechin gallate was slightly higher in Shiraz than Cabernet Sauvignon during fermentation, but decreased to around 4 % for both varieties after 12 months of aging.

After 12 months of aging, the composition of terminal subunits was similar for both varieties, with no epicatechin gallate detected. The terminal subunits catechin and epicatechin instead represented around 85 and 15 % respectively, irrespective of variety.

Table 3.5. Chemical analysis of small scale wines*.

	Shiraz			**Cabernet Sauvignon**		
	Pressing	Post fermentation	12 months aging	Pressing	Post fermentation	12 months aging
Total tannin (mg/L)	91.8 ± 5	117 ± 0.01	75.1 ± 0.002	193.1 ± 8	170.5 ± 0.01	77.7 ± 0.004
Average polymer length	10.1 ± 0.1	8.3 ± 0.08	5.8 ± 0.1	9.3 ± 0.2	5.5 ± 0.1	4.9 ± 0.1
Epigallocatechin extension	30.5 ± 0.1	29.6 ± 0.4	29.4 ± 1.3	33 ± 0.6	31 ± 0.6	28.7 ± 0.4
Catechin extension	2.1 ± 0.1	2.5 ± 0.04	2.2 ± 0.06	2.6 ± 0.04	2.5 ± 0.05	2.6 ± 0.2
Epicatechin extension	55.9 ± 0.6	54.9 ± 0.5	64.5 ± 1.2	56 ± 0.4	56.6 ± 0.5	64.6 ± 0.4
Epicatechin gallate extension	11.4 ± 0.9	13 ± 0.9	3.9 ± 0.1	8.4 ± 0.7	9.9 ± 0.3	4.2 ± 0.08
Catechin terminal	72 ± 1	68.1 ± 2.2	86 ± 0.6	73.7 ± 0.7	79.2 ± 0.6	84.2 ± 0.8
Epicatechin terminal	28 ± 1	21.5 ± 0.9	14 ± 0.6	17.8 ± 0.6	17.8 ± 0.6	15.8 ± 0.8
Epicatechin gallate terminal	n.d.	10.4 ± 1.3	n.d.	8.5 ± 0.8	3 ± 0.07	n.d.
Colour density (au)	-	9 ± 0.1	7.5 ± 0.04	-	2.2 ± 0.03	4.8 ± 0.1
Colour hue (au)	-	0.5 ± 0.003	0.6 ± 0.004	-	0.6 ± 0.003	0.7 ± 0.003
Total anthocyanins (mg/L)	-	438.1 ± 8	177.3 ± 3	-	201.4 ± 9	124.4 ± 6
Ionised anthocyanins (mg/L)	-	84.9 ± 2	38.3 ± 2	-	13.5 ± 0.4	16 ± 0.9
Total red pigments (au)	-	24.7 ± 0.4	13.5 ± 0.1	-	11.3 ± 0.4	9.6 ± 0.3
Total phenolics (au)	-	25.4 ± 0.4	13.3 ± 0.2	-	13.1 ± 0.7	8.4 ± 0.7

*Values are means of three replicates ± standard error n.d. = not detected

Red wine colour measurements made post fermentation and after 12 months of aging are shown for small scale wines in Table 3.5. Colour density was much lower for Cabernet Sauvignon than Shiraz. However, Shiraz colour density decreased from 8.9 au post fermentation to 7.5 au after 12 months of aging, but increased in Cabernet Sauvignon from 2.2 au post fermentation to 4.8 au after 12 months of aging. Colour

hue was slightly higher in Cabernet Sauvignon than Shiraz and increased slightly for both varieties between post fermentation and 12 months of aging. Shiraz had more than twice the total anthocyanin content of Cabernet Sauvignon post fermentation being 438 mg/L in Shiraz and 201 mg/mL in Cabernet Sauvignon. After 12 months of aging, total anthocyanin had decreased to 177 mg/L in Shiraz and 124 mg/L in Cabernet Sauvignon. Ionised anthocyanins in Shiraz decreased from 85 mg/L post fermentation to 38 mg/L after 12 months of aging and were much lower in Cabernet Sauvignon at around 15 mg/L post fermentation and 15 mg/L after 12 months of aging. Total red pigments decreased in Shiraz from 25 au post fermentation to 13 au after 12 months of aging, but lower levels were observed in Cabernet Sauvignon, being 11 au post fermentation and 9 au after 12 months of aging. Total phenolics also decreased for both Shiraz and Cabernet Sauvignon from post fermentation to 12 months of aging. Post fermentation, Shiraz contained 25 au total phenolics, while Cabernet Sauvignon was lower at 13 au. After 12 months of aging total phenolics levels were 13 au in Shiraz and 8 au in Cabernet Sauvignon.

Descriptive sensory analysis of small scale wines

Descriptive sensory analysis was conducted on Shiraz and Cabernet Sauvignon wines after 12 months of aging. The overall sensory profile (Figure 3.2.) was similar for both wines, with the exception of colour and colour intensity, which were significantly different ($p<0.05$). Interestingly, no significant differences were observed for any aroma or flavour descriptive terms.

Principal component analysis of sensory and chemical data is shown in Figure 3.3. Shiraz wines (112, 113 and 114) and Cabernet Sauvignon wines (203, 204 and 205) were separated primarily based on colour and colour intensity. Shiraz samples were associated more closely with ‘body’, ‘length’, ‘dark fruit’ and ‘spice’ attributes than

Cabernet Sauvignon samples, which were associated with 'astringency'. 'Light fruit', 'white pepper' and 'herbaceous' attributes were skewed more towards Cabernet Sauvignon than Shiraz wines.

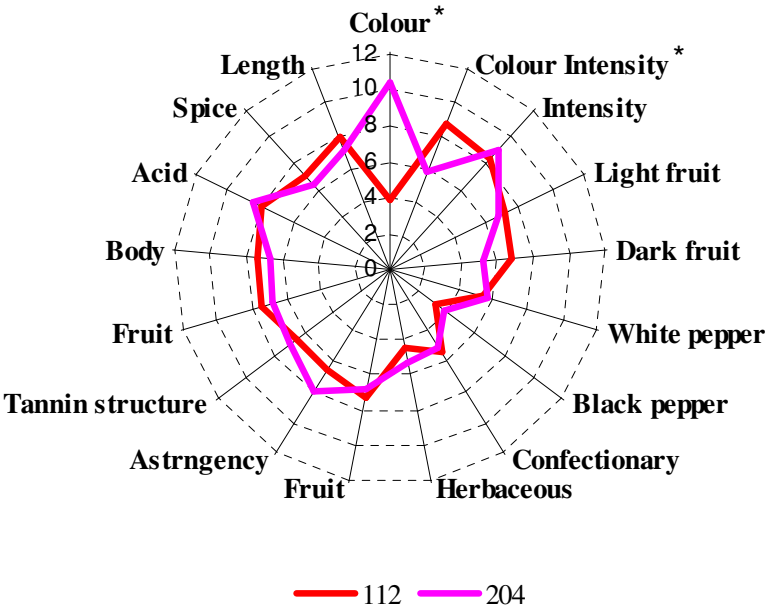

Figure 3.2. Descriptive sensory profile comparing Shiraz (112) and Cabernet Sauvignon (204) wine. Data is shown for 1 wine replicate. *Significant difference $p<0.05$.

The separation of Shiraz and Cabernet Sauvignon by colour was supported by chemical data with Shiraz associated with colour density and total anthocyanins, while Cabernet Sauvignon was associated with colour hue.

Interestingly, sensory descriptors associated with tannin did not necessarily correspond to chemical measures of tannin. The chemical measure of total tannin was most closely associated with the 'herbaceous' descriptor and polymer length with the 'spice' attribute. However, the mouthfeel descriptor 'length' was closely associated with total phenolics measurements. The attribute 'tannin structure' was slightly

skewed towards polymer length, while the descriptor 'astringency' was slightly skewed towards epicatechin gallate content.

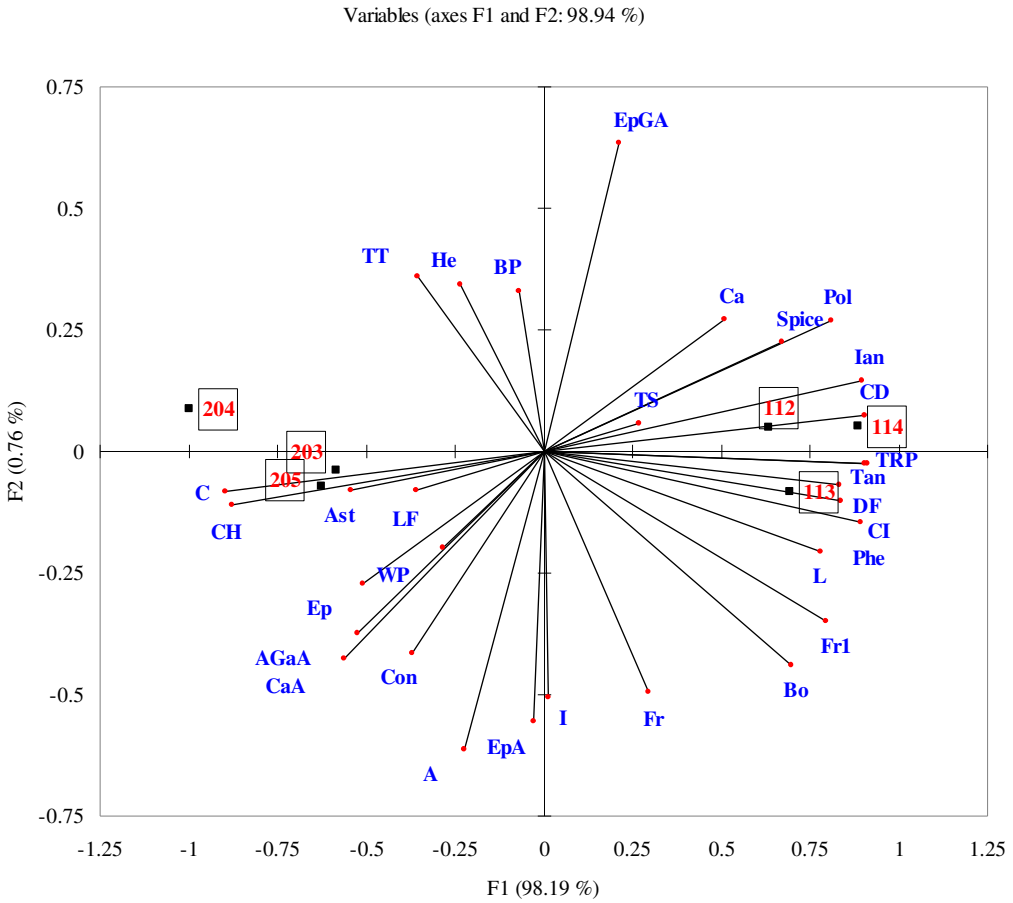

Figure 3.3. Principal component analysis of sensory and chemical data. Shiraz = 112, 113, 114. Cabernet Sauvignon = 203, 204, 205. Abbreviations: Colour = C, Colour Intensity = CI, Intensity = I, Light Fruit = LF, Dark Fruit = DF, White Pepper = WP, Black Pepper = BP, Confectionary = Con, Herbaceous = He, Fruit = Fr, Astringency = Ast, Tannin Structure = TS, Fruit = Fr1, Body = Bo, Acid = A, Length = L, Total Tannin = TT, Average Polymer Length = Pol, Epigallocatechin Extension = EpGA, Catechin Extension = CaA, Epicatechin Extension = EpA, Epicatechin gallate Extension = AGaA, Catechin Terminal = Ca, Epicatechin Terminal = Ep, Wine Colour Density = CD, Wine Colour Hue = CH, Total anthocyanins = Tan, Ionised anthocyanins = Ian, Total Red Pigments = TRP, Total phenolics = Phe.

DISCUSSION

This chapter describes an investigation into the content and composition of condensed tannin extracted from grapes into wine and the resulting impact on mouthfeel. To explore this, the tannin content and composition of grapes and wine was determined in grapes and then daily throughout micro-fermentation. Small scale wines were also made from the same grapes for which the tannin distribution was characterised in Chapter 2 to enable sensory descriptive analysis to be performed.

Tannin extraction during fermentation

The amount of tannin in the skin and seed components of grapes was similar for both Shiraz and Cabernet Sauvignon across all maturity levels. During micro-fermentation, similar amounts of tannin were also extracted into wine. Despite some variation in the results, there was no significant difference in tannin concentration between any samples at the end of fermentation, i.e. no apparent treatment effects. While not all tannin was extracted from grapes during fermentation, the results suggest that similar amounts of tannin were extracted from grapes of each variety using the standard winemaking protocol. The most significant difference observed between Shiraz and Cabernet Sauvignon samples, irrespective of maturity level, was tannin concentration at pressing. Cabernet Sauvignon wine samples had considerably higher levels of tannin than Shiraz samples, which suggests a difference in the rate of tannin extraction for the two varieties. Following pressing, the concentration of tannin decreased in Cabernet Sauvignon; this was also observed for Cabernet Sauvignon wines prepared according to the small scale winemaking protocol.

The difference in the rates of tannin extraction observed for Shiraz and Cabernet Sauvignon could be attributed to varietal differences in either the extraction or

solubility of tannin into wine. It seems that tannin is more readily extracted from Cabernet Sauvignon grapes than Shiraz. Given that Shiraz and Cabernet Sauvignon had similar tannin compositions, it is unlikely that tannin structure strongly influenced extraction. Rather, there must be another compositional difference influencing the tannin extraction rate of Cabernet Sauvignon compared with Shiraz. One possible hypothesis is variation in grape cell wall polysaccharide and protein composition, cell wall structure and cell wall thickness. Each of these factors could influence the affinity of tannin for the cell wall (*98*).

Differences in the structure and composition of cell wall polysaccharides between varieties may influence polysaccharide solubility into wine and therefore how quickly polysaccharides are broken down during fermentation. The concentration of grape derived polysaccharides, type II arabinogalactans and rhamnogalacturonan II, have been shown to increase during fermentation (*122, 123*). Grape maturity, variety and environmental conditions are also likely to influence the solubility and release of polysaccharides during fermentation (*98*). The concentration of protein in cell walls, which are also capable of binding tannin can also vary and might therefore influence tannin extraction (*45, 124*). Cell wall derived proteins are at their highest concentration in must at the commencement of fermentation, then decrease during winemaking (*122*). This decrease may also be partially responsible for the different rates of tannin extraction given that when protein binds tannin an insoluble complex will form.

The cell wall contains endogenous cell wall degrading enzymes, which represents another compositional difference that could influence the rate of tannin extraction. These enzymes can influence the breakdown of polysaccharides during fermentation,

which in turn can enhance the extraction of tannin. The activity of these enzymes have been shown to vary according to grape variety and maturity level (*125, 126*).

Morphological differences between varieties such as cell wall thickness and the amount of cell wall material present in skin and pulp can also influence the rate of tannin extraction (*110, 127*). Higher amounts of skin cell walls may indicate thicker skin cell walls, which may hinder the diffusion of tannin into the fermentation matrix. Once tannin is extracted from skin cell walls, it can then potentially bind to pulp derived cell walls already present in the fermentation matrix. As a consequence, higher amounts of pulp cell walls in the fermentation matrix may also increase the extent of polysaccharide-tannin binding and reduce the overall amount of tannin extracted into the wine.

To further investigate the influence of cell wall composition and tannin binding capacity on tannin extraction from Shiraz and Cabernet Sauvignon grapes at different maturity levels, the cell walls from grapes used in this chapter were characterised for polysaccharide composition and tannin binding capacity described in Chapter 4.

Descriptive sensory analysis of Shiraz and Cabernet Sauvignon wines

This chapter aimed to determine any correlations between the sensory properties of Shiraz and Cabernet Sauvignon wines and the grape and wine tannin content and composition determined in Chapter 2. Shiraz and Cabernet Sauvignon wines were rated for intensity of ‘dark fruit’, ‘light fruit’, ‘herbaceous’, ‘white pepper’, ‘black pepper’, ‘confectionary’ aromas and ‘fruit’ and ‘spice’ flavours, as well as ‘acid’, ‘astringency’, ‘tannin structure’, ‘body’ and ‘length’. No significant differences were perceived in the sensory attributes of Shiraz and Cabernet Sauvignon wines. Only the

visual descriptors of colour and colour intensity were found to be significantly different.

The colour differences detected between the wines were supported by chemical data, which showed higher levels for colour density and total anthocyanins in Shiraz wines compared to Cabernet Sauvignon wine. Despite Cabernet Sauvignon having higher levels of tannin post fermentation than Shiraz, the tannin content and composition of Cabernet Sauvignon and Shiraz was similar after 12 months of aging. The tannin distribution, as reported in Chapter 2, was also similar. This may explain why there was little difference observed in the sensory descriptors associated with tannin, i.e. 'astringency', 'tannin structure', 'length' and 'body'.

In earlier studies involving descriptive sensory analysis of wine, differences in the level of tannin post fermentation were maintained with wine aging. These studies reported lower perceived astringency in wines with lower concentrations of tannin (*88, 128, 129*). In the current study, the larger decrease in tannin concentration for Cabernet Sauvignon compared with Shiraz following aging might be related to the ratio of anthocyanin and tannin extracted into wine. It has been hypothesised that the ratio of tannin and anthocyanin in grapes and wine may play a key role in the formation and stability of pigmented polymers with aging (*129-131*). During fermentation, the higher concentration of anthocyanin in Shiraz might favour the formation of anthocyanin-tannin adducts, while the higher concentration of tannin in Cabernet Sauvignon might instead favour the formation of tannin-tannin adducts. However, with wine aging the stability and solubility of adducts might achieve an equilibrium in which the final tannin composition is similar for both wines.

It is also interesting to note that differences in anthocyanin concentration observed in this study had no influence on overall astringency. It has previously been reported that

anthocyanins can increase the astringent sensation of 'fullness' in model wine (*75*). Anthocyanin content has also been strongly correlated with the maximum intensity of astringency in wine (*132*). In this study, the descriptive term 'length' was most closely associated with total phenolics and total anthocyanins. These results suggest that although anthocyanins did not influence the overall perception of astringency, they could be involved in associated sensations such as 'fullness', 'length' and 'maximum intensity', which will have an impact on the overall mouthfeel and thus quality of wine. A more thorough investigations is required, but was beyond the scope of this research.

It is also unclear whether polymeric pigments contribute to perceived astringency (*49*). It has previously been reported that polymeric pigments contribute to perceived astringency (*49, 133*), however it was unclear whether the presence of tannin in the samples studied was responsible for the perception of astringency rather than pigmented polymers (*49, 133*). In this study, Shiraz had a higher concentration of total red pigments than Cabernet Sauvignon, but there was no difference in perceived astringency. This would suggest that pigmented polymers do not contribute to astringency.

The role of anthocyanin and polymeric pigments in the perception of astringency remains unclear. Further research is needed to characterise both the chemical structure and sensory properties of polymeric pigments and anthocyanins both individually and in combination with tannin. Further research is also needed to characterise the sensory properties of different tannin structures present in wine. While it is well established that higher levels of tannin correlate with an increase in the overall perceived astringency of wine (*118, 132, 133*), a decrease in the sensory descriptors 'coarse' and 'grainy' have been reported with a decrease in tannin concentration (*88, 129*).

However, these studies did not report tannin composition so it is not known whether the differences in perceived astringency were correlated to other structural differences of tannin, e.g. subunit composition or degree of polymerisation.

One of the major aims of this study was to investigate the influence of tannin structure on astringency by thoroughly characterising wine tannin distribution. However, the similarity in tannin distribution for Shiraz and Cabernet Sauvignon wines in this study did not provide opportunity for this aspect to be investigated adequately.

CONCLUSIONS

The rate of tannin extraction from grapes into wine differed for micro- and small scale wine fermentations of Shiraz and Cabernet Sauvignon must. Shiraz reached a maximum tannin concentration at the end of fermentation whereas Cabernet Sauvignon tannin levels reached maximum concentrations at pressing and decreased to similar levels as Shiraz by the end of fermentation. As tannin content and composition were similar in both varieties (for grapes and wine), it is likely that tannin composition did not strongly influence tannin extraction. Therefore, another factor, such as grape cell walls was likely the primary influence of tannin extraction and will be more thoroughly investigated in Chapter 4.

For small scale Shiraz and Cabernet Sauvignon wines, no significant differences were observed for sensory descriptors related to condensed tannins such as ‘astringency’, ‘tannin structure’, ‘body’ and ‘length’. Again, this is attributed to the similarity in tannin content and composition of Shiraz and Cabernet Sauvignon wines at the time of sensory analysis. Further research is needed to better determine the sensory properties imparted by different tannin structures in wine.

CHAPTER 4. CELL WALL COMPOSITION OF SHIRAZ AND CABERNET SAUVIGNON WINE GRAPES

INTRODUCTION

The measurement of tannin in the grape berry at harvest is not indicative of the level of tannin extracted into wine, which makes it difficult to arrive at informed winemaking decisions regarding wine style based on fruit composition. It is thought that the amount of tannin extracted into wine is reduced because the cell wall of the grape berry has the capacity to bind condensed tannins (*32*, *97*).

Varietal differences in the level of tannin extracted from grapes into wine have been attributed to differences in the tannin binding capacity of the cell wall material between these varieties (*32*). It is likely that variation in cell wall structure and composition will influence the tannin binding capacity of cell walls from different varieties.

Furthermore, the extraction of grape tannin into wine has also been observed to decrease during berry ripening and this has been attributed to cell wall remodelling that occurs as a part of the ripening process (*28, 29, 89*). While reduced tannin extraction during berry ripening might be a result of tannin becoming more physically entangled within the cell wall as structural modifications occur, changes in cell wall composition during this process may influence the binding capacity of the cell wall leading to differences in the extractability of tannin.

Grape cell wall structure and composition, together with interactions of tannins with cell walls has been extensively reviewed by Hanlin et al. (*98*).

The primary constituents of grape berry cell wall polysaccharides are glucosyl sugars that form the backbone of cellulose, arabinoxylans and xyloglucans that contain

xylosyl branching from the xyloglucan backbone of the latter polymer. The pectic polysaccharides, homogalacturonan and rhamnogalacturonan I and rhamnogalacturonan II have backbones consisting of galacturonosyl and rhamnogalacturonosyl residues with extensive branching from the rhamnogalacturonan backbones consisting primarily of arabinosyl, galactosyl and glycosyl residues. Arabinogalactan is also in abundance in grape berry cell walls as a major constituent of structural proteins (*34*).

Variation in the polysaccharide composition of grape skin and mesocarp has previously been observed between varieties and during maturation of grape berries. While the concentrations of cellulose and xyloglucan do not noticeably vary with berry ripening, the composition of pectic polysaccharides can vary substantially (*45*, *46*). Galacturonan, a primary component of the pectic polysaccharide backbone structure, has been observed to decrease during berry ripening in a number of grape varieties in both the skin and mesocarp (*37*, *45, 46*). While in grape skin, rhamnogalactan, which is a primary component of the rhamnogalacturonan I backbone, has been observed to increase slightly in Merlot, remain constant in Cabernet Sauvignon and decrease in Shiraz during grape berry ripening (*46*). In Monastrell skin, rhamnogalactan has been observed to both increase and decrease during ripening in fruit harvested from different vineyard locations (*46*). Variation in the level of arabinogalactan, a major component of the side chains of both rhamnogalacturonan I and rhamnogalacturonan II, has also been observed during berry ripening with increases in some grape varieties, but no compositional changes in other varieties (*45*, *46*).

Despite considerable variation in cell wall composition having been previously reported in wine grapes, no direct comparison has been made between cell wall

composition and its tannin binding capacity. Therefore, the aim of this chapter was to determine whether or not a link exists between cell wall composition, the tannin binding capacity of cell walls and the amount of tannin extracted into Shiraz and Cabernet Sauvignon wine made from fruit harvested at three different maturity levels.

MATERIALS AND METHODS

Sample collection and cell wall preparation

Shiraz and Cabernet Sauvignon grape berries were harvested at three maturity levels as described in the Materials and Methods section of Chapter 3. Approximately 2 kg of frozen grape bunches were de-stemmed and a subset of 100 berries were de-seeded and stored at -80^{o}C until needed for whole berry cell wall analysis. For skin cell wall analysis, the remaining berries were thawed and skinned at 4 ^{o}C by expulsion of the seeds and flesh. Skins were then stored at -80 ^{o}C until analysed.

For cell wall preparations, frozen grape skins (50 g) or de-seeded whole berries (100 berries) were ground using an IKA grinder (All Basic grinder, IKA Works, Petaling Jaya, Malaysia) then placed immediately into a beaker on ice. The ground material was suspended in 200 mL of absolute ethanol then filtered sequentially through nylon mesh with pore sizes of 350, 280 and 71 μm using 80 % (v/v) aqueous ethanol to wash the solids. Material retained on the 71 μm mesh was stirred for 45 minutes at room temperature in 50 mL of saturated phenol with 500 mM Tris-HCl buffer (pH 7.0) (*134*). The suspension was filtered through a single layer of Miracloth (Calbiochem, Merck, Australia) and washed with 80 % (v/v) aqueous ethanol and 100 % (v/v) acetone to remove phenol. The retained solids were suspended in 150 mL of chloroform:methanol (1:1, v/v), stirred for one hour and vacuum-filtered through a

sintered-glass funnel (Grade 1 pore size). The solids were re-suspended in 150 mL of chloroform:methanol (1:1, v/v) and filtered twice. The solids were then suspended in 150 mL of 90 % aqueous acetone (v/v), stirred for one hour and filtered through a sintered glass funnel (Grade 1 pore size). The retained solids were dried in a vacuum oven at 25^{o}C overnight and stored over silica gel in a vacuum desiccator. Cell wall isolates were prepared in duplicate.

Microscopy

Following preparation, skin cell wall isolates were examined by scanning electron microscopy. Isolated cell walls were mounted on metal stubs and coated with platinum. Samples were examined in a Philips XL30 scanning electron microscope (FEI, Oregon, USA) at the University of Adelaide Microscope Centre (Adelaide, Australia) using an accelerating voltage of 10 kV.

Polysaccharide carboxyl reduction

To distinguish between neutral, uronic and methylated sugars, uronic acids and esterified uronic acids of the duplicate cell wall preparations were reduced by carboxyl reduction prior to polysaccharide linkage analysis according to the method of Kim and Carpita (*135*). Cell wall samples (5 mg) were suspended in imidazole-HCl buffer (5 mL, 1 M, pH 7.0). Esterified uronic acids were reduced on ice by three sequential additions of sodium borodeuteride (1 mL, 100 mg/mL in water) at 5 minute intervals, with vortexing (10 seconds). Following the third addition, samples were incubated for 2 hours on ice. Excess sodium borodeuteride was then destroyed with glacial acetic acid (approx. 500 μL, 100 %). Samples were dialysed for 16 hours against Milli-Q water (6,000-8,000 molecular mass cut-off) and freeze-dried. Samples were then resuspended in Milli-Q water (1 mL) and MES buffer (200 μL, 0.2 M, pH

4.75). Free uronic acid residues were derivatised by adding carbodiimide (400 µL, 500 mg/mL in water), vortexed (10 seconds) and incubated for 3 hours at 30°C. Samples were then cooled on ice and imidazole-HCl buffer (1 mL, 4 M, pH 7.0) was added. The samples were then split in two and had either sodium borodeuteride (1 mL, 70 mg/mL) added for the determination of total uronic acids or sodium borohydride (1 mL, 70 mg/mL) to determine the proportion of uronic acids that were esterified. The two sets of samples were incubated for 3 hours at room temperature (~23°C). Following incubation, excess reductant was destroyed by adding glacial acetic acid (approx 500 µL, 100 %). The preparations were then dialysed for 24 hours against Milli-Q water (6,000-8,000 molecular mass cut-off) and freeze dried.

Polysaccharide linkage analysis

To determine the position of sugar linkages, methylation of both sets of carboxyl reduced cell walls was conducted by the method of Ciucanu and Kerek (*136*). Following carboxyl reduction, the dried sample was resuspended in Milli-Q water (1 mL) and an aliquot (100 µL) was freeze dried. The dried aliquot was then dissolved in dimethylsulfoxide (100 µL, 100 %) and sonicated for 20 minutes at room temperature (22°C). For methylation, each sample had sodium hydroxide [100 µL, 120 mg/mL in 100 % (v/v) dimethylsulfoxide] added prior to sonication for 20 minutes. Two sequential additions of methyl iodide (20 µL, 100 %) were made to each sample with 10 minutes sonication following each addition. Another 40 µL of methyl iodide (100 %) was then added prior to a further 20 minutes sonication. Milli-Q water (1 mL) and dichloromethane (1 mL, 100 %) were added, samples were vortexed (40 seconds) and centrifuged (5 minutes, 1250 x g) to separate the phases. The aqueous phase was removed and the remaining organic phase was washed three times with Milli-Q water (1 mL) by vortexing (15 seconds) and centrifuging (5 minutes, 1250 x g). The

aqueous layer was removed following each wash and the remaining organic phase was dried under nitrogen.

Following methylation, samples were hydrolysed to cleave the polysaccharides into individual sugar constituents. For hydrolysis, trifluoroacetic acid (100 µL, 2.0 M) was added to the methylated sample and incubated for 90 minutes at 121^{o}C. Following hydrolysis, samples were cooled in a water bath (~30^{o}C) and evaporated to dryness by flushing with nitrogen. *Myo*-inositol (2.5 µg) was added as an internal standard and the sample was dried by flushing with nitrogen.

Following hydrolysis, sugars were reduced and acetylated to partially methylated alditol acetates that were analysed by gas chromatography mass spectrometry (GCMS). Hydrolysed samples were reduced by dissolving the dried sample in ammonia (50 µL, 2 M) and adding sodium borodeuteride (50 µL, 1 M in 2 M ammonia). The sample was then sonicated for 1 minute and incubated at room temperature (22^{o}C) for 2.5 hours. Excess reductant was destroyed with glacial acetic acid (20 µL, 100 %), and samples dried by flushing with nitrogen. The sample was then washed twice with acetic acid [250 µL, 5 % (v/v) in methanol] followed by washing twice with methanol (250 µL, 100 %). The sample was dried by flushing with nitrogen following each wash.

For acetylation, acetic anhydride (250 µL, 100 %) was added to the sample, sonicated for 5 minutes (22^{o}C) and incubated for 2.5 hours at 100^{o}C. Excess acetic anhydride was destroyed by adding Milli-Q water (2 mL), mixing and standing for 10 minutes at room temperature (22^{o}C). Partially methylated polysaccharides were extracted in dichloromethane (1 mL, 100 %), vortexed (40 seconds) and centrifuged (5 minutes, 1250 x g) to separate the phases. The aqueous layer containing excess acetic anhydride was removed and the organic layer with the partially methylated

polysaccharides was washed twice with Milli-Q water (1 mL). The aqueous layer was removed following each wash and the remaining organic layer was dried by flushing with nitrogen. The dried sample was then redissolved in dichloromethane (20 µL, 100 %) and analysed by GC-MS. Partially methylated alditol acetates were separated on a high polarity BPX70 column using conditions described by Lau and Bacic (*137*). Neutral sugar and uronic acid derivatives were identified and quantified using the method described by Lau and Bacic (*137*).

Tannin binding capacity of cell walls

A standardised grape seed tannin extract was prepared by sonicating 50 g of whole Chardonnay grape seeds in 300 mL of 70 % (v/v) aqueous acetone for 1 hour at room temperature (~23^{o}C). Tannin extract was collected by vacuum filtration through Whatman #1 filter paper, concentrated under vacuum at 30^{o}C to remove acetone and freeze dried to remove water. A standard tannin solution (1 mg/mL) was prepared in water.

For cell wall material, frozen grape skin (10 g) was ground using an IKA grinder and placed immediately in 150 mL of 70 % (v/v) aqueous acetone. For whole berry cell wall material, seeds were removed and discarded from 20 berries. The remaining flesh and skin were weighed, frozen in liquid nitrogen and ground using an IKA grinder, then placed into 150 mL of 70 % (v/v) aqueous acetone.

Both skin and whole berry material were stirred for 2.5 hours, then filtered by vacuum filtration through Whatman #1 filter paper to collect insoluble cell wall material. The insoluble cell wall material was washed with 70 % (v/v) aqueous acetone, weighed and resuspended in 40 mL of Milli-Q water. A 500 µL aliquot of cell wall suspension was centrifuged in a 1.5 mL Eppendorf test tube and the water removed by pipette.

The remaining cell wall material was weighed and adjusted to 20 mg by removing excess cell wall material with a spatula.

To determine the tannin binding capacity of the cell wall, the tannin standard (1 mL, 1 mg/mL) was added to the weighed cell wall material (20 mg) and incubated at room temperature (~23^{o}C) for 20 minutes with vortexing (5 seconds) every 5 minutes. The cell wall material was then centrifuged (5 minutes, 3000 x g) and a 100 µL aliquot of the supernatant containing tannin that did not bind to the cell wall material was dried under reduced pressure at room temperature (~23^{o}C). A 100 µL aliquot of fresh tannin standard was also dried for the determination of the tannin concentration prior to cell wall binding. The dried tannin standard and tannin from the supernatant then underwent acid-catalysed cleavage in the presence of phloroglucinol to determine total tannin, subunit composition and average polymer length following the methods described by Hanlin and Downey (*89*).

The amount of tannin bound by the cell wall was determined by calculating the difference in tannin concentration of the standard tannin starting material, and the unbound tannin remaining in the supernatant following cell wall binding and centrifugation of the tannin-cell wall complex.

RESULTS

Histological examination of grape berry cell walls

Scanning electron microscopy of skin cell wall preparations from Shiraz and Cabernet Sauvignon grapes are shown in Figure 4.1. Fragments of the cell wall preparations show skin cell walls that were composed of thick and compact layers of cell wall material. Fragments for all of the maturity dates for Cabernet Sauvignon (Figure 4.1a-

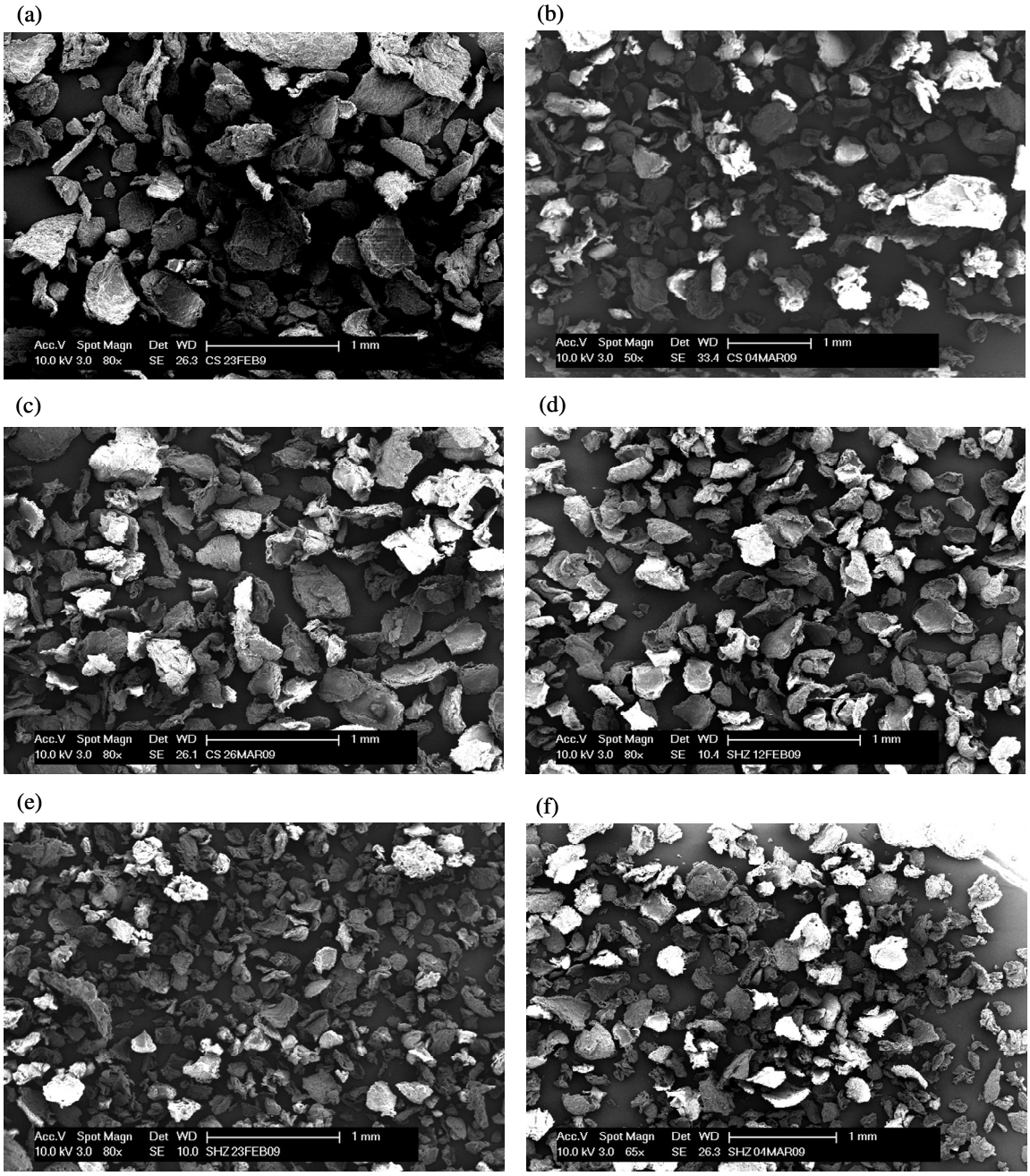

Figure 4.1. Scanning electron micrograph of isolated skin cell walls derived from Cabernet Sauvignon grapes harvested on the (a) 23rd February 2009, (b) 4th March 2009 and (c) 26th March 2009, and derived from Shiraz grapes harvested on the (d) 12th February 2009, (e) 23rd February 2009 and (f) 4th March 2009.

4.1c) appeared slightly larger than those for Shiraz (Figure 4.1d-4.1f). Closer magnification of the cell wall fragments showed little difference between the individual samples. The surfaces of cell walls were smooth, rippled surfaces

with compact layers visible (Figure 4.2a, only Cabernet Sauvignon from the 26th March 2009 shown). The cell walls themselves were thick, with crumpling and folding observed between layers (Figure 4.2b). Further magnification of cell wall fragments showed knobbly features on the cell wall surface and adherence of some cytoplasmic material (Figure 4.2c).

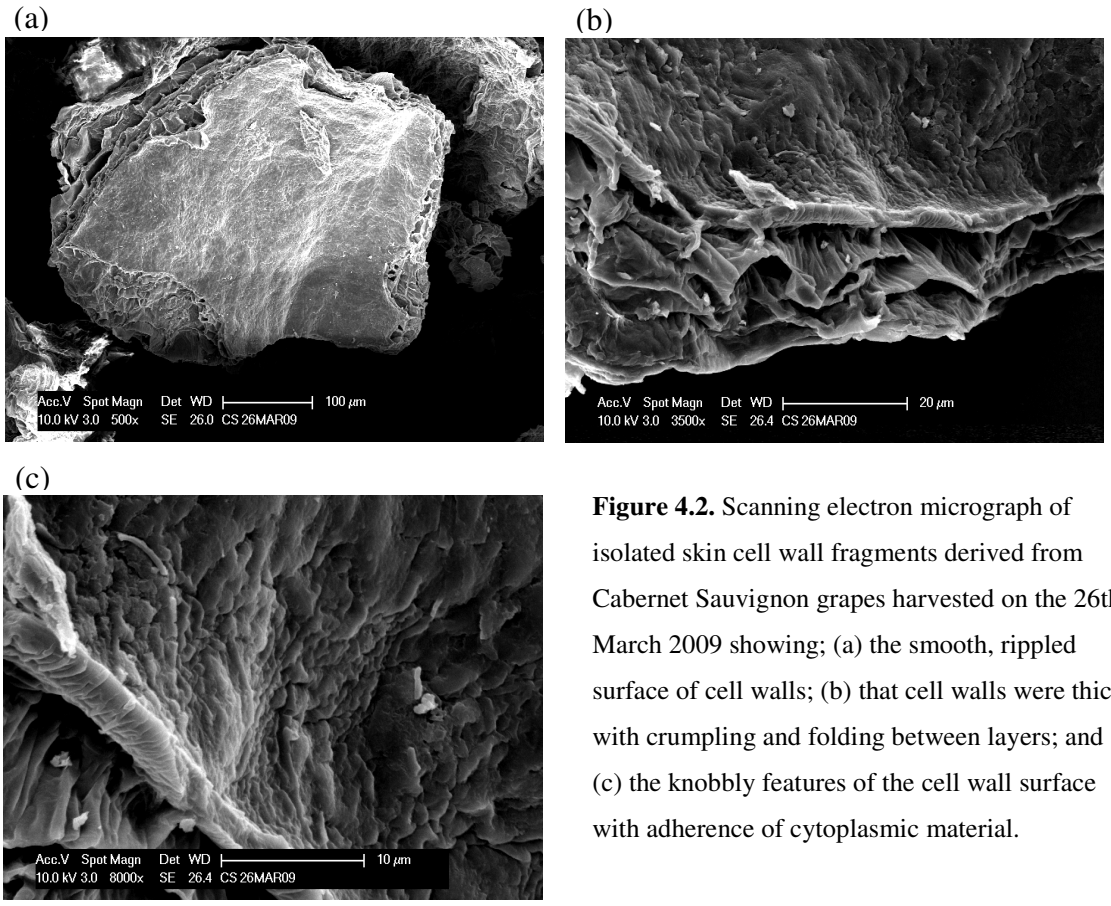

Figure 4.2. Scanning electron micrograph of isolated skin cell wall fragments derived from Cabernet Sauvignon grapes harvested on the 26th March 2009 showing; (a) the smooth, rippled surface of cell walls; (b) that cell walls were thick with crumpling and folding between layers; and (c) the knobbly features of the cell wall surface with adherence of cytoplasmic material.

Polysaccharide linkage analysis

The monosaccharide composition and linkage of grape cell wall polysaccharides was determined by carboxyl reduction, methylation and GCMS analysis of cell wall preparations. For both Shiraz and Cabernet Sauvignon grape skin, the monosaccharide present in the highest proportion was (1,4)-linked D-glucopyranose, followed by (1,4)-

linked D-galacturonic acid, (1,4)-linked D-galacturonic acid esters and L-arabinofuranose. Other monosaccharides and their linkages present in grape skin are shown in Table 4.1.

Table 4.1. Monosaccharide linkage composition (mol %) of cell walls isolated from skins of Shiraz and Cabernet Sauvignon grapes harvested at three different maturity levels*.

Monosaccharide linkage composition of skin cell walls (mol %)

		Shiraz			Cabernet Sauvignon		
Sugar	**Linkage**	12-Feb-09	23-Feb-09	4-Mar-09	23-Feb-09	14-Mar-09	26-Mar-09
L-Rhamnopyranose	Terminal	1	tr	1	1	2	2
	2	1	1	1	2	1	1
	2,4	1	1	1	1	1	1
L-Fucopyranose	Terminal	1	1	1	1	1	1
α-L-Arabinopyranose	Terminal	1	1	1	1	1	1
L-Arabinofuranose	Terminal	11	8	7	10	8	9
	5	3	3	4	5	2	5
D-Xylopyranose	Terminal	3	3	4	3	3	3
	4	5	4	5	8	7	6
	2	1	1	1	1	1	1
	2,4	1	1	1	2	1	1
	2,3,4	3	1	1	2	2	1
D-Mannopyranose	4	4	4	4	4	5	4
	4,6	tr	1	1	1	1	1
D-Glucopyranose	Terminal	1	1	1	tr	tr	1
	4	33	46	40	24	37	34
	3,4	1	1	1	tr	1	1
	2,4	tr	tr	tr	tr	tr	tr
	4,6	3	4	4	3	3	3
D-Galactopyranose	Terminal	2	2	2	3	2	2
	3	1	1	tr	1	1	1
	2	1	1	2	3	2	1
	4	-	tr	-	tr	-	-
	6	tr	tr	tr	1	tr	1
	3,4	1	tr	1	1	1	tr
	2,4	tr	tr	tr	tr	tr	tr
	3,6	3	2	1	2	2	2
D-Glucuronic acid	Terminal	1	1	1	2	2	1
	4	-	-	tr	-	tr	tr
D-Galacturonic acid	4	4	5	4	7	5	6
Methylated D-galacturonic acid	4	10	7	8	11	8	9

*tr = trace (<1 mol %), n = 4

In Shiraz skin, the monosaccharide (1,4)-linked D-glucopyranose increased and decreased with increasing maturity, lowest in abundance (expressed as % in proportion) at the earliest harvest date representing 33 % in proportion, increasing to 46 % at the second harvest date and decreasing to 40 % at the final harvest. The proportion of (1,4)-linked D-glucopyranose followed a similar pattern in Cabernet Sauvignon, representing 24 % at the earliest harvest date, increasing to 37 % at the second harvest date and decreasing to 34 % at the final harvest date. The combined total of the monosaccharide D-galacturonic acid was around 13 % of all monsaccharides in Shiraz and 16 % in Cabernet Sauvignon at all harvest dates. Approximately 70 % of the D-galacturonic acid monosaccharides were esterified. Of the L-arabinofuranose monosaccharides, the terminal L-arabinofuranose residues were present in both Shiraz and Cabernet Sauvignon skin at around 8 to 11 % of all monosaccharides while the (1,5)-linked L-arabinofuranose represented 2 to 5 % of all monosaccharides. In both Shiraz and Cabernet Sauvignon skin, the terminal D-xylopyranose monosaccharide residue composed around 3 % of all monsaccharides, while the (1,4)-linked D-xylopyranose was around 5 % in Shiraz. In Cabernet Sauvignon, (1,4)-linked D-xylopyranose was slightly higher representing around 6 to 8 % of all monosaccharides. The (4,6)-linked D-glucopyranose represented around 3 to 4 % of monosaccharide residues in both varieties and (1,4)-linked D-mannopyranose represented around 4 %. The remaining monosaccharide residues were low in proportion representing less than 3 % in both varieties.

In de-seeded Shiraz and Cabernet Sauvignon berries (ie. grape flesh and skin with the seeds removed), the monosaccharide residue (1,4)-linked D-glucopyranose was highest in proportion followed by (1,4)-linked D-galacturonic acid, (1,4)-linked D-galacturonic acid esters and L-arabinofuranose. Berry cell wall monosaccharides and

their linkages are presented in Table 4.2. In Shiraz berries, (1,4)-linked D-galacturonic acid decreased with maturity from 23 % at the earliest harvest date to 15 % at the final harvest date. In Cabernet Sauvignon berries, the proportion of (1,4)-linked D-

Table 4.2. Monosaccharide linkage composition (mol %) of cell walls derived from Shiraz and Cabernet Sauvignon grape berries (skin and flesh with seeds removed) at three harvest dates*.

Monosaccharide linkage composition of berry cell walls (mol %)

		Shiraz			Cabernet Sauvignon		
Sugar	**Linkage**	12-Feb-09	23-Feb-09	4-Mar-09	23-Feb-09	14-Mar-09	26-Mar-09
L-Rhamnopyranose	Terminal	1	1	1	1	1	1
	2	1	2	2	1	1	1
	2,4	1	1	1	tr	tr	tr
L-Fucopyranose	Terminal	1	1	1	tr	1	1
α-L-Arabinopyranose	Terminal	tr	2	1	1	-	-
L-Arabinofuranose	Terminal	13	12	12	11	10	11
	5	3	4	3	3	3	3
D-Xylopyranose	Terminal	5	5	6	2	5	4
	4	8	9	11	10	8	8
	2	1	1	1	1	1	1
	2,4	2	2	2	2	1	2
	2,3,4	1	2	tr	1	1	tr
D-Mannopyranose	4	6	5	6	8	7	5
	4,6	1	1	-	1	1	tr
D-Glucopyranose	Terminal	-	tr	1	-	-	1
	4	23	16	15	17	28	20
	3,4	tr	-	-	-	-	-
	2,4	tr	tr	-	tr	-	-
	4,6	3	3	2	2	3	3
D-Galactopyranose	Terminal	2	2	2	2	2	2
	3	2	1	2	2	1	1
	2	-	-	-	-	-	-
	4	-	-	-	-	-	-
	6	tr	tr	1		tr	-
	3,4	1	1	1	1	1	1
	2,4	tr	tr	tr	tr	-	tr
	3,6	4	4	4	3	3	2
D-Glucuronic acid	Terminal	2	2	2	3	2	1
	4	-	tr	tr	-	-	tr
D-Galacturonic acid	4	9	11	11	14	10	15
Methylated D-galacturonic acid	4	9	11	11	14	10	15

*tr = trace (<1 mol %), n = 4

galacturonic acid varied representing 17 % at the earliest harvest date, increasing to 28 % at the second harvest and decreasing to 20 % at the final harvest. The combined total for the monosaccharide D-galacturonic acid residues represented between 20 and 30 % in Shiraz and Cabernet Sauvignon berries with approximately 50 % esterification. Of the L-arabinofuranose monosaccharides, the terminal residue represented between 10 and 13 % of both Shiraz and Cabernet Sauvignon berries while the (1,5)-linked L-arabinofuranose represented 3 %. In both Shiraz and Cabernet Sauvignon berries, the terminal D-xylopyranose monosaccharide residue composed between 2 and 6 %, while the (1,4)-linked D-xylopyranose was around 8 to 11 % in both varieties. The (4,6)-linked D-glucopyranose represented 2 and 3 % in both varieties and (1,4)-linked D-mannopyranose represented between 4 and 8 %. The remaining monosaccharide residues were low in proportion representing less than 3 % in both varieties.

Polysaccharide composition

The polysaccharide composition of grape skin and berry cell walls was deduced from the linkage analysis shown in Table 4.1 and 4.2. These polysaccharide compositions were determined from the total mol percentage (mol %) of individual glycosyl residues that are characteristic of well defined cell wall polysaccharides (*138, 139*).

Arabinan was calculated from the amount of (1,5)-linked L-arabinofuranose present in the sample; Type I arabinogalactan was accounted for by (3,4)-linked D-galactopyranose and the terminal α-L-arabinopyranose with (3,4)-linked D-galactopyranose branch point; Type II arabinogalactan was determined by summing the (1,3)-, (1,6)- and (3,6)-linked D-galactopyranose, together with the terminal L-arabinofuranose with a (3,6)-linked D-galactopyranose branch point; Glucuroarabinoxylan was estimated by summing (1,4)-, (2,4)- and (2,3,4)-linked D-

xylopyranose with the equivalent terminal L-arabinofuranose or D-glucuronic acid with a branch point of (2,4)- and (3,4)-linked D-xylopyranose and two (2,3,4)-linked D-xylopyranose; Xyloglucan was estimated by summing (1,2)-linked D-xylopyranose, equal amounts of (4,6)- and (1,4)-linked D-glucopyranose, terminal D-xylopyranose, terminal L-fucopyranose and terminal D-galactopyranose with the sum of the terminal residues equal to (4,6)-linked D-glucopyranose. The (1,4)-linked D-glucopyranose not accounted for by xyloglucan was assigned to cellulose; Galactomannan was estimated by summing (1,4)- and (4,6)-linked D-mannopyranose with the terminal D-galactopyranose equal to (4,6)-linked D-mannopyranose; Pectin was determined as the sum of (1,4)-linked D-galacturonic acid, (1,2)- and (2,4)-linked L-rhamnopyranose. Any remaining linkages that were not accounted for were classified as 'other'.

The deduced polysaccharide composition of cell walls isolated from Shiraz and Cabernet Sauvignon skin and berries are shown in Table 4.3 and 4.4. For grape skin, cellulose was present in the highest proportion in both varieties, followed by pectin, and the hemicelluloses, glucuronoarabinoxylan and xyloglucan. Cellulose was lowest

Table 4.3. Polysaccharide composition (mol %) of cell walls isolated from skin of Shiraz and Cabernet Sauvignon deduced from the monosaccharide linkage analysis.

Polysaccharide composition of skin cell walls (mol %)

	Shiraz			Cabernet Sauvignon		
Polysaccharide	12-Feb-09	23-Feb-09	4-Mar-09	23-Feb-09	14-Mar-09	26-Mar-09
Arabinan	4	3	4	5	2	4
Type I Arabinogalactan	1	1	1	2	1	1
Type II Arabinogalactan	8	4	3	6	4	5
Glucuronoarabinoxylan	16	10	11	19	15	11
Xyloglucan	10	10	11	9	10	10
Galactomannan	5	5	5	6	7	6
Pectin	16 (70)[a]	14 (61)	15 (65)	21 (61)	15 (60)	17 (60)
Cellulose	30	42	36	21	33	31
Other	10	11	14	11	13	15

[a]= % esterification shown in brackets

in both varieties at the earliest harvest date, representing 30 % in Shiraz skin and 21 % in Cabernet Sauvignon skin. The proportion of cellulose had increased by the following harvest date representing 42 and 33 % in Shiraz and Cabernet Sauvignon respectively, but then decreased to represent 36 and 31 % for Shiraz and Cabernet Sauvignon, respectively by the final harvest date. Pectin represented between 14 and 21 % of the skin cell walls in Shiraz and Cabernet Sauvignon with around 65 % esterification. Xyloglucan represented around 10 % of the polysaccharides in skin cell walls at all three harvest dates for both varieties, while the proportion of glucuronarabinoxylan was highest at the earliest harvest date, representing 16 % of the cell wall in Shiraz and 19 % in Cabernet Sauvignon. The proportion of glucuronarabinoxylan decreased with maturity in both varieties representing 11 % in both Shiraz and Cabernet Sauvignon skin at the final harvest date. In grape skin, Type I arabinogalactan represented only 1 and 2 % of the cell wall in both varieties, while Type II arabinogalactan represented between 3 and 8 % in both varieties, decreasing slightly with maturity. Arabinan represented between 2 and 5 % in Shiraz and

Table 4.4. Polysaccharide composition (mol %) of Shiraz and Cabernet Sauvignon whole berry (skin and flesh with seeds removed) cell walls deduced from the monosaccharide linkage analysis.

Polysaccharide composition of berry cell walls (mol %)

	Shiraz			Cabernet Sauvignon		
Polysaccharide	12-Feb-09	23-Feb-09	4-Mar-09	23-Feb-09	14-Mar-09	26-Mar-09
Arabinan	3	4	3	3	3	3
Type I Arabinogalactan	2	3	3	2	2	2
Type II Arabinogalactan	10	9	11	8	7	5
Glucuronoarabinoxylan	16	19	17	16	14	13
Xyloglucan	9	10	8	6	8	8
Galactomannan	8	6	6	10	9	6
Pectin	19 (50)[a]	25 (50)	25 (50)	29 (50)	22 (50)	32 (50)
Cellulose	20	13	12	15	25	17
Other	13	11	15	11	10	14

[a]= % esterification shown in brackets

Cabernet Sauvignon skin, while galactomannan represented between 5 and 7 % of skin cell wall polysaccharides. In the whole grape berry (i.e. flesh and skin with seeds removed), pectin was highest in proportion. Cellulose and glucuronarabinoxylan were also present in high proportionsfollowed by Type II arabinogalactan, galactomannan and xyloglucan.

Pectin varied with maturity in both varieties representing between 19 and 25 % in Shiraz and 22 and 32 % in Cabernet Sauvignon with 50 % esterification. Cellulose decreased with maturity in Shiraz berries from 20 % at the earliest harvest date to 12 % at the final harvest. However, in Cabernet Sauvignon berries, the proportion of cellulose varied with maturity representing 29 % at the earliest harvest date, decreasing to 22 % at the second harvest date then increasing to the highest proportion being 32 % at the final harvest date. Glucuronoarabinoxylan varied between 16 and 19 % in Shiraz berries and 13 and 16 % in Cabernet Sauvignon berries. Type I arabinogalactan represented around 2 to 3 % in both Shiraz and Cabernet Sauvignon berries, while Type II arabinogalactan represented 9 to 11 % in Shiraz and 5 to 8 % in Cabernet Sauvignon. Arabinan represented around 3 % of berry polysaccharides in both varieties while galactomannan represented 6 to 10 % in Shiraz and Cabernet Sauvignon berry cell walls.

Tannin binding capacity of cell walls

The capacity of cell wall material to bind condensed tannin was determined for both skin and berry cell walls. The amount of cell wall material and tannin binding capacity in Shiraz and Cabernet Sauvignon at different maturity levels is reported in Table 4.5.

The amount of cell wall material increased with maturity in Shiraz skin from 40 mg per berry at the earliest harvest date to 60 mg per berry at the final harvest. The amount of cell wall material was highest in Cabernet Sauvignon skin at the final

Table 4.5. The amount of cell wall material (shown as mg of cell wall material per berry) and the tannin binding capacity of cell wall material (determined as µg of tannin bound per mg of cell wall material) for cell walls isolated from Shiraz and Cabernet Sauvignon grape skin and berries at three harvest dates*.

	Shiraz			Cabernet Sauvignon		
	12-Feb-09	23-Feb-09	4-Mar-09	23-Feb-09	14-Mar-09	26-Mar-09
Amount of skin cell wall material (mg per berry)	40 ± 5	41 ± 6	60 ± 5	37 ± 1	27 ± 2	44 ± 3
Amount of berry cell wall material (mg per berry)	52 ± 4	47 ± 6	63 ± 12	51 ± 2	67 ± 2	55 ± 2
Tannin binding capacity of skin cell walls (µg/mg of cell wall material)	3.59 ± 0.2	3.48 ± 0.2	3.78 ± 0.4	2.68 ± 0.2	2.98 ± 0.2	2.91 ± 0.2
Tannin binding capacity of berry cell walls (µg/mg of cell wall material)	3.14 ± 0.1	3.48 ± 0.2	3.11 ± 3.1	5.07 ± 0.2	4.73 ± 0.1	3.71 ± 0.2

*Values are means of three replicates ± standard error

harvest date and lowest at the second harvest date, ranging between 27 and 44 mg per berry. The amount of cell wall material in berries with combined skin and flesh varied for both varieties. Cell wall material in Shiraz berries ranged from 47 to 63 mg per berry and was highest at the final harvest. In Cabernet Sauvignon the amount of cell wall material ranged from 51 to 67 mg per berry and was highest at the second harvest date.

For Shiraz, the binding capacity of cell walls was similar for both skin and berry cell wall material. The binding capacity of Shiraz cell wall material ranged between 3.11 and 3.78 µg of tannin per mg of cell wall material. The binding capacity of Cabernet Sauvignon skin cell wall material was slightly lower than Shiraz cell wall material, ranging between 2.68 and 2.91 µg of tannin per mg of cell wall material. The tannin

binding capacity of Cabernet Sauvignon berry cell walls decreased with maturity from 5.07 to 3.71 μg of tannin per mg of cell wall material.

DISCUSSION

The aim of this chapter was to determine whether or not a link exists between the cell wall composition of grape berries, the tannin binding capacity of cell walls and the amount of tannin extracted into wine. Cell walls were isolated from the skin and whole berries (skin and flesh with the seeds removed) of Shiraz and Cabernet Sauvignon grapes harvested at three different maturity levels to investigate the effect of grape maturity on the structure, composition and tannin binding capacity of cell walls.

Skin cell wall shape and structure

Skin cell wall preparations derived from Shiraz and Cabernet Sauvignon grapes were analysed by scanning electron microscopy to determine if there were any obvious physical differences in the structure of skin cell walls. Isolated cell wall fragments were generally uniform in size and were slightly larger for Cabernet Sauvignon than Shiraz. The thickness and smooth surface of cell wall fragments were similar to cell walls isolated from skins of Monastrell grapes (*140*). Shiraz cell wall fragments were generally smaller than Cabernet Sauvignon fragments, which might be related to the strength and structure of cell walls at the time of preparation. The latter conclusion may indicate differences in the cell wall structure that make Cabernet Sauvignon cell walls more rigid and less resistant to homogenisation compared with Shiraz cell walls. Higher magnification of the cell walls did not elucidate any apparent differences in cell wall structure between Shiraz and Cabernet Sauvignon or any obvious physical

changes occurring as a result of increased grape maturity. Generally, cell walls were stacked together in thick, slightly ruffled layers. The thickness of cell walls was 100 nm or less, which is typical of parenchyma cell walls in higher plants (*138, 141*). However, compared to electron micrographs of cell walls isolated from the mesocarp of Muscat Gordo Blanco grape berries (*42*), cell walls in the skin appeared more rigid and thicker. The skin cell walls were stacked together in thick layers, while the cell walls isolated from mesocarp appeared to be more loosely clumped and extensively folded and crumpled (*42*). The differences observed between skin and mesocarp cell walls are most likely due to skin cell walls acting as a protective barrier during berry development.

Polysaccharide composition and tannin binding capacity

In Chapter 3, the tannin content of Shiraz and Cabernet Sauvignon fermentations were monitored throughout winemaking to quantify tannin extraction from grapes into wine. It was found that there was no significant difference in the amount of tannin at the end of fermentation. However there were significant differences in the rates of tannin extraction between varieties; in particular, at pressing after 7 days of fermentation on skins. It was concluded that the presence of cell walls was likely influencing the rate of tannin extraction. To investigate this further, the polysaccharide composition of cell walls and their tannin binding capacity were determined. Specifically, the aim of this chapter was to investigate potential links between cell wall composition, the tannin binding capacity of the cell walls and the amount of tannin extracted into wine.

Both skin and whole berry (composed of skin and flesh with the seeds removed) samples were analysed to evaluate the role of individual grape components in tannin and cell wall interactions during fermentation. As tannin is located in the skin of the

grape berry, it is initially extracted from the skin into the fermentation matrix. Once extracted from the skin, tannin may subsequently interact with other fermentation components such as polysaccharides derived from grape flesh (mesocarp), present in the fermentation matrix.

The monosaccharide composition and linkage of polysaccharides in cell walls isolated from skin and whole berry samples were determined by carboxyl reduction, methylation and GCMS. The monosaccharide composition and linkage of polysaccharides were similar to that previously reported for grape cell walls (*42, 45*, *46, 126*). These results were then used to estimate the oligosaccharide composition of skin and whole berry derived cell walls isolated from Shiraz and Cabernet Sauvignon grapes harvested at three maturity levels, to provide a more accurate representation of the cell wall structure.

In Shiraz skin, the proportion of the different oligosaccharides was similar to that reported previously in grape skin (*38, 40*). Cell walls were primarily composed of cellulose, followed by pectin, ranging between 30 and 42 mol % and 14 and 16 mol % respectively. The non-cellulosic polysaccharides, xyloglucan, glucuronoarabinoxylan and galactomannan were also present in significant proportions. Type II arabinogalactan ranged between 3 and 8 mol %, with Type I arabinogalactan and arabinan composing around 1 and 4 mol % respectively.

While the oligosaccharide present in the largest proportion in Shiraz skin was cellulose, pectin was the oligosaccharide that was present in the largest proportion in whole Shiraz berries. In Shiraz berries, pectin ranged between 19 and 25 mol %, while cellulose ranged between 12 and 20 mol %. The larger proportion of pectin in whole berries is consistent with the presence of flesh material, which had a higher proportion of pectin compared to skin (38, *41, 42*). There was also a higher proportion of the

hemicellulose, glucuronarabinoxylan present in whole Shiraz berries composing between 16 and 19 mol %. Type II arabinogalactan was also present in slightly higher proportions in whole Shiraz berries compared to Shiraz skin, representing between 9 and 10 mol %. The higher proportion of Type II arabinogalactan in whole berries was consistent with the higher proportion of pectin as it is thought that Type II arabinogalactan is present in branches of the pectic polysaccharide, rhamnogalacturonan I (*34*).

In Cabernet Sauvignon, the proportions of oligosaccharides were similar to those observed in Shiraz. Again, cellulose was present in the highest proportion in Cabernet Sauvignon skin, while pectin was present in the highest proportion in whole berries. However, both Cabernet Sauvignon skin and whole berries had slightly higher proportions of pectin than was observed in Shiraz. An earlier study found similar levels of pectin in Shiraz and Cabernet Sauvignon skin during ripening, but Monastrell grape skin had higher levels of pectin (*46*). The differences in the levels of pectin between varieties have been attributed to the firmer cell wall structure of skin that is composed of more pectin (*46*). It has been suggested that the more rigid skin cell wall structure may reduce the amount of tannin extracted during winemaking (*126*). However, in the current study, the higher proportion of pectin found in Cabernet Sauvignon compared with Shiraz did not influence the amount of tannin extracted during winemaking (Chapter 3).

Variation was also observed in the proportions of pectin and cellulose in both Shiraz and Cabernet Sauvignon skins and whole berries at different maturities. The proportion of these oligosaccharides both increased and decreased during maturity with no clear trend. Previous analysis of polysaccharide composition during berry

ripening has shown that the levels of different polysaccharides increase, decrease or remain constant in the skin and flesh of grapes (*45, 46*).

The variation in the samples reported here and the lack of any correlation with earlier studies could indicate variability and limitations in the methodology, rather than the absence of any underlying trend. It is difficult to obtain complete methylation of grape skin samples due to the presence of contaminating material such as tannins that interfere with the analysis of cell walls. Despite washing the cell wall material with acetone during the cell wall preparation to remove phenolic material, this solvent system [ie. 90 % (v/v) aqueous acetone] is not ideal for the complete extraction of tannin from the cell wall material. The optimum solvent system for tannin extraction from grape skin is 70 % (v/v) aqueous acetone (*142*). However, cell wall sugars are soluble in water and the cell wall preparation also becomes susceptible to the growth of mould at higher aqueous levels making 70 % (v/v) aqueous acetone unsuitable for cell wall preparation. During the method development for this work, scanning electron microscope analysis was carried out on cell wall preparations utilising a range of aqueous acetone mixtures. Mould was observed to contaminate cell wall preparations containing less than 90 % aqueous acetone (data not shown). Therefore, the optimum solvent for removing tannin material was not suitable for cell wall preparations.

Despite difficulties in performing complete methylation of skin and whole berry cell wall preparations, the analyses were repeated where poor methylation was observed to increase the confidence in the results reported.

Given the variation in cell wall composition, variation was expected for measurements of tannin binding capacity, but was not observed for Shiraz skin or whole berry samples. The tannin binding capacity of Shiraz skin and whole berries was similar at around 3.5 µg of tannin per mg of cell wall material for all maturity

levels. The variation in cell wall composition for Shiraz skin and whole berry cell walls did not correlate with the tannin binding capacity.

For Cabernet Sauvignon, the tannin binding capacity of cell walls isolated from skins was also similar across all maturity levels being around 2.9 μg of tannin per mg of cell wall material. This was slightly lower than the tannin binding capacity of Shiraz skin cell wall samples. However, for Cabernet Sauvignon whole berry samples, the tannin binding capacity of cell walls decreased with maturity, from around 5 μg of tannin per mg of cell wall material to 3.7 μg of tannin per mg of cell wall material. The decrease in tannin binding capacity did not correlate with any trend in cell wall composition of Cabernet Sauvignon whole berries.

It has previously been reported that tannin has a higher affinity for pectin than the non-cellulosic polysaccharides or cellulose (*78*). In the samples analysed in this study, Cabernet Sauvignon had a slightly higher proportion of pectin than Shiraz, however, Shiraz skin cell walls showed a slightly higher tannin binding capacity than Cabernet Sauvignon skin cell walls. In Cabernet Sauvignon whole berries, pectin content was highest at the latest maturity level, however, tannin binding capacity decreased with maturity for Cabernet Sauvignon whole berries.

Although no link was observed between cell wall composition and the tannin binding capacity of cell walls, the analysis of monosaccharide composition was limited to the measurement of individual sugars and gives no indication of cell wall structural features that might influence its tannin binding capacity. Further research of cell wall structure, such as polysaccharide size, cell wall thickness and density may elucidate cell wall features which better explain the tannin binding capacity of cell walls.

In an earlier study, the thickness of skin cell walls was thought to influence the amount of tannin extracted into wine (*126*). Diffusion of tannin from skin cell walls

into the fermentation matrix is likely to take longer with thicker cell walls. Higher amounts of skin cell wall material may indicate thicker cell walls in skin and higher amounts of cell wall material in the flesh may indicate an increase in the potential of the fermentation matrix to bind tannin once it has been extracted from the skin and seeds. In this study, the amount of cell wall material was determined for both skin and whole berries. The amount of skin cell wall material was higher for Shiraz skin than for Cabernet Sauvignon, which suggests that Shiraz skin had thicker cell walls than Cabernet Sauvignon. That Cabernet Sauvignon had thinner cell walls would suggest tannin would be extracted more quickly, which was supported by the faster rate of tannin extraction observed for Cabernet Sauvignon during micro-fermentation experiments described in Chapter 2. At pressing of micro-ferments, ie. when the skins were removed from fermentation, Cabernet Sauvignon samples had higher levels of tannin than corresponding Shiraz samples. Following pressing, tannins may bind to soluble polysaccharides that are present in the fermentation matrix. The amount of soluble polysaccharides in the fermentation might be determined by the amount of cell wall material present in the whole berry. In this study, it was observed that the amount of cell wall material isolated from whole berries was similar for both Shiraz and Cabernet Sauvignon. However, Cabernet Sauvignon whole berry cell walls were composed of a higher proportion of cell walls derived from flesh. The higher proportion of flesh cell walls in Cabernet Sauvignon compared to Shiraz may indicate a higher amount of soluble cell wall material in the fermentation matrix capable of binding larger amounts of tannin. This may explain the decrease in tannin observed following pressing of the Cabernet Sauvignon micro-fermentions (Chapter 3).

CONCLUSIONS

The composition of cell walls varied during maturation for both Shiraz and Cabernet Sauvignon, but with no observable trend. Despite variation in the cell wall composition, the tannin binding capacity of skin cell walls was similar at all maturity levels. Slightly more pectin was present in Cabernet Sauvignon samples compared to Shiraz samples, however, the higher proportion of pectin did not correlate with a higher tannin binding capacity of Cabernet Sauvignon cell walls. Whole berries were also composed of more pectin than skin samples, but again did not result in a higher tannin binding capacity. No obvious link was found between cell wall composition and the tannin binding capacity of cell walls.

Shiraz grapes contained more skin cell wall material than Cabernet Sauvignon grapes indicating thicker skin cell walls were present in Shiraz. The thicker skin cell walls of Shiraz suggests more time would be required for tannin to diffuse through the cell wall, thus decreasing the rate of tannin extraction, which was consistent with the observation in micro-ferments in Chapter 2. Further, a higher proportion of flesh cell wall material in Cabernet Sauvignon may have the capacity to bind more tannin, thus reducing the amount of tannin in the final wine. Both skin and flesh cell wall material are likely to bind tannins and therefore to influence the amount of tannin remaining in the final wine.

CHAPTER 5. A COMPARISON OF THE TANNIN DISTRIBUTION AND TANNIN BINDING CAPACITY OF CELL WALLS IN SKINS OF SHIRAZ WINE GRAPES GROWN UNDER A RANGE OF ENVIRONMENTAL CONDITIONS

INTRODUCTION

The viticultural production environment varies by region according to climate, soil and topography as well as under different vineyard management practices, all of which influence vineyard microclimate. Variation in environmental conditions such as temperature, light, soil, humidity, altitude, rootstock and canopy vigour can all influence the many biosynthetic pathways involved in the synthesis of tannin, its precursors, cell wall components and their subsequent modifications. Collectively, these factors influence a) the final concentration, composition and polymer length distribution of tannin and b) the composition and structure of cell wall polysaccharides in the grape berry.

A range of factors including altitude, water potential, fertiliser application, rootstock, vine vigour and light exposure have been shown to affect tannin concentration (*87, 88, 92, 143-146*). Vine vigour and light exposure appear to have the most significant impacts on tannin synthesis, with significantly lower maximum tannin concentrations reported for shaded fruit compared to exposed fruit during berry development (*145*). Higher concentrations of tannin have been reported in the skin of mature grapes harvested from low vigour vines (*88, 92*). Variation in tannin concentration has also been reported in the skin of Pinot Noir grapes grown on different rootstocks independent of the effect of rootstock on vine vigour (*146*).

Tannin composition has been found to vary considerably between growing seasons and regions (*28, 29, 89, 91, 94*). While studies to date have indicated that the proportion of the extension subunit epigallocatechin is typically lower in cool climate growing regions, the proportion of epigallocatechin can increase or decrease in the skins of Shiraz and Cabernet Sauvignon grapes from one season to another (*89*). Grapes grown in the Sunraysia region have been shown to contain a high proportion of epigallocatechin ranging from 25 to 54 % compared to cooler climates for which epigallocatechin ranges between 10 and 35 % (*15, 26, 28, 29, 91, 94, 147*). The regional variation in grape skin epigallocatechin content has been hypothesised to be a function of differences in the temperature and light exposure of cooler and warmer climates (*89*). Previous research has shown the proportion of epigallocatechin decreases with increased shading and vigour (*92, 145*).

The influence of environmental conditions on tannin polymer length is unclear as most tannin related studies have been unable to correlate growing conditions and average tannin polymer length. One study reported that temperature had no effect on polymer length distribution at harvest as determined by gel permeation chromatography (*148*), but no other studies have investigated the influence of environmental factors on polymer length distribution.

Yet, differences in tannin distribution may influence the amount of tannin that is extracted into wine and final wine quality.

As discussed in earlier chapters, the amount of tannin extracted from grapes into wine during fermentation is influenced by the tannin binding capacity of cell walls (*32, 97*). Different environmental conditions are likely to change the structure and composition of the cell walls within grapes. It has been reported that cell wall composition such as the proportion of pectic polysaccharides varies between vineyards and seasons (*44,*

46, 149). Differences in cell wall structure may change the tannin binding capacity of cell walls thereby influencing the amount and potential composition of tannin extracted into wine. To date, no studies have been undertaken to investigate the influence of viticultural and environmental factors such as temperature, light or canopy vigour on the structure and composition of cell walls in grapes.

In Chapter 3, it was discussed that the ratio of anthocyanin to tannin extracted from grapes into wine could play a role in the stability of tannin during wine aging. During red winemaking and aging, tannins and anthocyanins react to form more stable polymeric pigments (*51*). These reactions can occur by direct tannin-anthocyanin reactions or reactions involving acetaldehyde (*51*). Direct reactions involve tannins and anthocyanins reacting as nucleophiles and electrophiles, and vice versa, to form anthocyanin-tannin adducts and tannin-anthocyanin adducts respectively (*61*). In reactions involving acetaldehyde, anthocyanin and tannins are linked by an ethyl bridge arising from acetaldehyde mediated condensation (*6, 150*).

The extent to which these reactions occur during either winemaking or aging are likely to be influenced by the ratio of anthocyanin to tannin. Given tannin and anthocyanin content in grapes is influenced by various environmental conditions (*87*), the ratio of anthocyanin to tannin will vary by site, season and management practices all of which could determine the aging potential of wine derived from different regions.

The aim of this chapter was to investigate the extent to which several common environmental conditions influence skin tannin distribution of Shiraz grapes and tannin binding capacity of cell walls isolated from skins of Shiraz grapes. The tannin distribution, tannin binding capacity of cell walls and amounts of tannin and anthocyanin extracted into wine were determined in a) the skin of Shiraz grapes

harvested from low, medium and high canopy vigour vines on Schwarzmann rootstock and b) in the skin of Shiraz grapes harvested from vines grown on Paulsen rootstock and on own roots, each sourced from a vineyard located in Sunraysia (Victoria, Australia). Shiraz grapes haversted from vines grown on Schwarzmann rootstock were also sourced from the cooler growing region of Glenrowan (Victoria, Australia).

METHODS

Sample collection

Shiraz grape bunches were collected at commercial maturity in the 2010 season from two southeastern Australian wine regions. Five Shiraz samples were collected from a vineyard located in Sunraysia, northwest Victoria (34°27'S,142°14'E). Three samples were collected from low, medium and high canopy vigour sections of a block that had been previously mapped to determine canopy vigour (*151*) on the 22nd of February 2010. Grapes from the medium vigour vines were harvested from the same panels harvested in the 2009 season described in Chapter 2 to enable seasonal comparison of tannin composition. The low, medium and high vigour vines were all PT23 Shiraz clones grown on Schwarzmann rootstock. The fourth sample comprising PT23 Shiraz vines grown on Paulsen rootstock in an adjacent block was randomly collected on the 26th of February 2010. The fifth sample was harvested from vines grown on their own roots comprising a combination of PT23 and DVRC12 Shiraz clones on the 26th of February 2010.

A sixth sample was collected from PT23 vines grown on Schwarzmann rootstock in a vineyard located in Glenrowan, southeast Victoria on the 19th of February 2010.

Whole grape bunches (approximately 100 kg) were collected randomly from 10 panels of grapevines for each sample. Two sub-samples (2 kg each) were stored at -20^{o}C to enable skin tannin analysis and cell wall analysis to be undertaken at a later date. The remaining grape bunches were stored at -20^{o}C for one week prior to small scale winemaking.

Concentration, composition and polymer length distribution of skin tannin

Skin tannin was isolated according to the sample preparation and extraction protocols described in Chapter 2. Skin tannin was fractionated using semi-preparative diol phase chromatography and analysed by UV-Vis spectrophotometry, phloroglucinolysis and high performance liquid chromatography (HPLC) techniques.

The total tannin concentration was determined by HPLC as both the sum of the individual subunit concentrations calculated using the conversion factors relative to catechin (*26*) and as the sum of each individual subunit calculated as catechin equivalents. The tannin polymer length or degree of polymerisation (DP) was determined by dividing the sum of extension subunits and terminal subunits by the total of terminal subunits following determination of the concentration using conversion factors for each subunit relative to catechin. The percent conversion yield to subunits following phloroglucinolysis was determined as the proportion of the total tannin concentration determined by HPLC catechin equivalents compared to the total tannin concentration determined by UV-Vis absorbance (A_{280}) prior to phloroglucinolysis as catechin equivalents. Semi-preparative fractions comprising the same DP were pooled to report tannin concentration, percent conversion yield, and the proportion of extension and terminal subunits at individual DP values.

Cell wall analysis and tannin binding capacity

Skin cell walls were isolated to determine the amount of cell wall material (mg of cell wall material per g of grape skin) isolated from grape skin and its tannin binding capacity following the methods described in Chapter 4. The tannin binding capacity of cell walls was determined by adding a known concentration of a tannin standard extracted from grape seeds to the isolated cell wall material to determine the amount of tannin that remained bound to cell wall material. The amount of tannin bound by the cell wall was determined by calculating the difference in tannin concentration determined by HPLC and phloroglucinolysis for the individual tannin standard and the unbound tannin remaining in the supernatant following cell wall binding and centrifugation of the tannin-cell wall complex following incubation.

Winemaking

Each of the six Shiraz grape samples were made into wine (in triplicate, 25 kg scale) at the CSIRO small scale winemaking facility in Merbein (Victoria, Australia) using the protocols described in Chapter 2.

Prior to fermentation, a sub-sample of 100 berries was collected to enable grape skin and seed tannin content and composition to be determined using the methods described by Hanlin and Downey (*89*). Wine tannin content and composition were determined at the end of fermentation using the methods described in Chapter 3.

Anthocyanin analysis

For anthocyanin analysis of grapes, grape skins were collected prior to fermentation of small scale wines (100 berry sub-sample) by expulsion of the seeds and flesh. The skins were immediately frozen in liquid nitrogen and ground to a fine powder using

an IKA grinder (All Basic grinder, IKA Works, Petaling Jaya, Malaysia). Samples were stored at -80°C until analysed.

Extraction and HPLC analysis of anthocyanins from grape skin were performed in triplicate following the method described by Downey and Rochfort (*152*).

Wine anthocyanins were determined at the end of fermentation following the same method, but without sample preparation. A 200 μL aliquot of wine was centrifuged (5 minutes, 16,100 x g) then transferred to a HPLC vial prior to analysis.

Wine colour and co-pigmentation analysis

Red wine colour and co-pigmentation of small scale wines were measured using a micro-plate spectrophotometer (SpectraMax Plus384 Absorbance Microplate reader, Molecular Devices, Sunnyvale, USA) and polystyrene flat bottom 96 well plates (Greiner Bio-One, Frickenhausen, Germany). Red wine colour parameters included wine colour density, wine hue, total anthocyanins, ionised anthocyanins, total red pigments and total phenolics, and were determined using the methods developed by Somers and Evans (*119*) and Iland et al. (*120*). Co-pigmentation parameters including percentage of colour due to anthocyanins, co-pigmentation complex and polymeric pigments, and were determined using the methods developed by Levengood (*153*) and Lambert (*154*).

Statistical analysis

Total skin and seed tannin, skin and wine anthocyanin concentration and wine colour and co-pigmentation data from samples used in small scale winemaking were analysed by analysis of variance (ANOVA) using Genstat software (13th edition).

RESULTS

DP range and distribution

The DP range and distribution of Shiraz grape skin tannin was determined by calculating the DP of each fraction and summing the concentrations of fractions with the same DP.

The DP of Shiraz skin tannin derived from grapes grown on vines of low, medium and high vigour canopies and on Schwarzmann rootstock ranged from 4 to 45 subunits (Table 5.1, 5.2 and 5.3).

The pattern of skin tannin distribution at different DP was similar to that reported in Chapter 2, with DP below 16 subunits representing less than 6 % of the total tannin concentration. The DP with the highest concentrations occurred above DP 16.

For Shiraz grapes harvested from low vigour vines, DP was reported at 17 values with the highest concentrations calculated by HPLC occurring at DP 20, 35 and 38 with each representing around 15 % of the total concentration of all DP. When calculated by UV-Vis spectrophotometry, DP 20 had the highest concentration of skin tannin in grapes grown on low vigour vines, representing around 13 % of the total concentration at all DP.

For Shiraz skin tannin from grapes grown on medium vigour vines, the DP with the highest concentration calculated by HPLC was DP 20 representing around 15 % of the concentration for all DP, followed by DP 31 representing 14 %. Calculated by UV-Vis spectrophotometry, DP 20 also had the highest concentration representing 13 % of all DP in skin tannin from medium vigour vines.

Calculated by HPLC, the DP with highest concentration for Shiraz grapes grown on high vigour vines was DP 41 representing around 17 % of the total concentration,

while the DP with the highest concentration for high vigour vines when calculated by UV-Vis spectrophotometry was DP 15 representing around 15 %.

For Shiraz skin tannin from grapes grown on Paulsen rootstock, the DP ranged between 3 and 46 subunits (Table 5.4). Calculated by HPLC, skin tannin from grapes grown on Paulsen rootstock had the highest concentration at DP 41 representing around 17 %. When calculated by UV-Vis spectrophotometry, the DP with the highest concentration for skin tannin from Shiraz on Paulsen rootstock was DP 14 representing 17 % followed by DP 24, which represented 13 %.

For Shiraz skin tannin from vines grown on own roots, the DP ranged between 4 and 46 with the highest concentration occurring at DP 37 representing 20 % when calculated by HPLC (Table 5.5). Calculated by UV-Vis spectrophotometry, the highest concentration for own roots skin tannin occurred at DP 16 representing 16 % of the total concentration at all DP.

For Shiraz skin tannin from grapes grown on Schwarzmann rootstock in the cooler growing region of Glenrowan, the DP ranged between 4 and 51 subunits (Table 5.6). When calculated by HPLC, the highest concentration occurred at DP 47 and 48 representing 16 % at both DP values. DP 20 and 33 represented 15 and 14 % of the total concentration respectively. Calculated by UV-Vis spectrophotometry, DP 20 had the highest concentration for Glenrowan Shiraz skin tannin representing 14 % of the total concentration followed by DP 33 and 4, which represented 13 and 11 % respectively.

The total skin tannin concentration determined as the sum of the concentration reported at all DP was lowest for grapes grown on Schwarzmann rootstock in the cooler growing region of Glenrowan with a total concentration of 1711.4 mg/L. The total skin tannin concentration was highest in grapes grown on Schwarmann rootstock

with high vigour at 2209.8 mg/L and grapes grown on Paulsen rootstock at 2207.3 mg/L and on own roots at 2189.8 mg/L. Grapes grown on Schwarzmann rootstock on medium and low vigour vines had lower skin tannin concentrations than grapes grown on high vigour vines at 1826.1 and 1863.0 mg/L respectively.

In addition to the total concentration at each DP, the percent conversion yield was also calculated (Tables 5.1-5.6). The percent conversion yield increased with increasing DP for all samples. At a DP of 4, the percent conversion yield was low at around 4 to 7 % for all samples. The percent conversion yield steadily increased in all samples to around 25 to 28 % by DP 11 to 15. Above DP 30, the percent conversion yield increased to above 30 %. For grapes grown on low and high vigour vines, the conversion yield reached a maximum of 40 % at DP 44, while grapes grown on medium vigour vines reached 48 % conversion yield at DP 43.

Shiraz skin tannin from grapes grown on Paulsen and own roots reached the highest percent conversion yields of 54 % at DP 45 and 53 % at DP 39, while Glenrowan Shiraz skin tannin reached a maximum percent conversion yield of 44 % at DP 47.

Extension subunit composition

The extension subunit composition was determined as the average composition at each DP (Tables 5.1-5.6). Epigallocatechin, catechin, epicatechin and epicatechin gallate were all detected as extension subunits.

The extension subunit composition of Shiraz skin tannin was similar for all samples. The extension subunit epicatechin was present in the highest proportion representing between 55 and 79 % for grapes grown in Sunraysia and 50 to 75 % for grapes grown in Glenrowan. The proportion of epicatechin decreased slightly as DP increased.

The extension subunit epigallocatechin was present in the second highest proportion and increased with increasing DP. The proportion of epigallocatechin was highest in

Shiraz skin tannin from grapes grown in Glenrowan and increased from 16 to 42 % as DP increased. For grapes grown in Sunraysia, the proportion of epigallocatechin also increased with increasing DP, but a slightly lower proportion was observed at low DP with a minimum between 11 and 15 % and a maximum between 35 and 37 % at high DP. While catechin and epicatechin gallate were also present as extension subunits in all Shiraz skin tannin samples, they only represented 2 and 7 % of all extension subunits respectively across all DP.

Terminal subunit composition

The terminal subunit composition was determined by calculating the average terminal subunit composition at each DP. Catechin and epicatechin were present as terminal subunits in the skins of all Shiraz samples (Tables 5.1-5.6). Epicatechin gallate and epigallocatechin were not detected as terminal subunits.

Catechin was present in higher proportions than epicatechin for all samples. The proportion of catechin in the skin of grapes from low vigour vines ranged between 56 and 86 % while epicatechin ranged between 14 and 44 % of all terminal subunits. For skin of grapes from medium vigour vines, the proportion of catechin and epicatechin ranged between 44 and 85 % and 15 and 56 % respectively. Epicatechin levels were only greater than catechin at DP 34.

In Shiraz grape skin from high vigour vines, the proportion of terminal subunit catechin was always much higher than epicatechin. Catechin level ranged between 77 and 89 %, while epicatechin ranged between 11 and 23 %.

The composition of terminal subunits in grape skin tannin was similar for Shiraz grown on Paulsen and own roots with catechin most abundant at between 60 and 92 % of terminal subunits and epicatechin representing between 8 and 40 %. For Glenrowan Shiraz skin tannin, the proportion of terminal subunit catechin ranged between 70 and

Table 5.1. Distribution of tannin extracted from skin of Shiraz grapes harvested from low vigour canopy vines grown on Schwarzmann rootstock sourced from Sunraysia (Victoria, Australia).

	Total tannin concentration				% Conversion	% of extension subunits				% of terminal subunits	
DP	(mg/L HPLC)[a]	(% by HPLC)[b]	(% by HPLC CE)[c]	(% by UV-Vis CE)[d]	**yield**	Epigall	Cat	Ecat	Ecatgall	Cat	Ecat
4	23.5	1.3	1.4	7.7	4.0	15.4	2.4	75.1	7.0	70.1	29.9
5	17.9	1.0	1.1	4.3	5.7	16.5	1.9	73.9	7.7	73.0	27.0
6	33.1	1.8	2.0	5.6	7.6	16.9	1.6	74.2	7.3	74.2	25.8
7	51.1	2.7	3.1	6.0	11.2	15.9	1.5	74.7	7.8	75.7	24.3
8	54.4	2.9	3.3	6.5	11.2	16.6	1.5	73.8	8.1	75.2	24.8
9	50.5	2.7	3.0	6.0	11.2	17.9	1.8	72.2	8.0	74.2	25.8
10	32.2	1.7	1.9	3.9	11.0	18.2	1.9	71.6	8.3	72.5	27.5
11	17.0	0.9	1.0	1.9	11.7	17.5	1.4	72.8	8.3	72.3	27.7
13	23.8	1.3	1.4	1.2	25.1	18.6	1.1	72.5	7.7	83.2	16.8
16	142.8	7.7	8.2	6.3	28.5	24.2	0.8	67.4	7.7	86.3	13.7
20	284.4	15.3	15.7	13.4	25.5	32.4	1.0	58.0	8.5	82.7	17.3
35	267.8	14.4	13.9	10.9	28.2	36.2	1.0	56.0	6.7	77.2	22.8
38	279.2	15.0	14.2	8.9	38.6	35.8	0.9	57.7	5.5	63.6	36.4
39	174.8	9.4	9.0	5.4	36.4	35.6	0.9	58.0	5.6	68.1	31.9
42	75.3	4.0	3.8	2.1	39.9	35.2	0.9	58.7	5.2	56.5	43.5
44	227.4	12.2	11.6	6.7	40.3	35.2	0.8	58.6	5.4	64.2	35.8
45	107.8	5.8	5.5	3.2	37.0	34.8	0.8	59.0	5.4	63.2	36.8
Total (mg/L)	1863.0										
					Total tannin extract						
31					15.3	39.7	2.2	52.5	5.7	56.3	43.7

Abbreviations: Epigall = epigallocatechin, Cat = Catechin, Ecat = Epicatechin, and Epicatgall = Epicatechin gallate. [a]Total tannin concentration at each DP following HPLC/phloroglucinolysis determined by the summation of individual subunit concentrations determined using conversion factors relative to catechin. [b]The proportion of the total tannin concentration for all DP calculated from the tannin concentration following HPLC/phloroglucinolysis and the calculation of individual subunits using conversion factors relative to catechin. [c]The proportion of the total concentration for all DP determined following HPLC/phloroglucinolysis and calculated as catechin equivalents. [d]The proportion of the total concentration for all DP determined by absorbance at 280 nm on a UV-Vis spectrophotometer before phloroglucinolysis.

Table 5.2. Distribution of tannin extracted from skin of Shiraz grapes harvested from medium vigour canopy vines grown on Schwarzmann rootstock sourced from Sunraysia (Victoria, Australia).

	Total tannin concentration				% Conversion	% of extension subunits				% of terminal subunits	
DP	(mg/L HPLC)[a]	(% by HPLC)[b]	(% by HPLC CE)[c]	(% by UV-Vis CE)[d]	**yield**	Epigall	Cat	Ecat	Ecatgall	Cat	Ecat
4	33.9	1.9	2.1	8.9	6.1	13.9	2.1	76.6	7.5	68.8	31.2
5	43.5	2.4	2.7	8.7	8.4	14.6	1.6	75.4	8.4	72.3	27.7
6	25.5	1.4	1.6	3.8	11.6	12.8	1.3	77.2	8.7	72.4	27.6
7	79.7	4.4	5.0	10.0	13.4	14.2	1.6	75.5	8.7	70.9	29.1
8	103.8	5.7	6.4	12.2	14.0	15.3	1.6	74.5	8.7	62.8	37.2
11	22.4	1.2	1.4	1.3	28.0	15.5	1.3	74.6	8.6	75.4	24.6
15	133.5	7.3	8.0	5.4	38.2	22.1	0.6	69.1	8.2	85.8	14.2
20	280.9	15.4	16.1	13.8	30.4	30.1	1.2	59.8	8.9	83.1	16.9
31	264.2	14.5	14.1	10.3	35.6	35.4	1.0	56.7	6.9	74.3	25.7
34	59.4	3.3	3.0	2.0	39.1	36.9	1.0	57.5	4.6	44.6	55.4
36	74.4	4.1	3.7	2.9	33.4	36.8	1.0	57.9	4.4	50.5	49.5
38	97.3	5.3	4.9	3.1	40.9	36.7	0.8	58.0	4.4	52.7	47.3
40	69.7	3.8	3.5	2.3	39.3	37.2	0.9	57.5	4.4	47.8	52.2
41	224.4	12.3	11.6	6.4	47.4	37.3	0.8	56.0	5.9	69.3	30.7
43	176.6	9.7	9.0	4.8	48.5	37.5	0.8	56.2	5.4	65.2	34.8
47	136.9	7.5	7.0	3.9	46.2	37.5	0.8	56.4	5.3	63.2	36.8
Total (mg/L)	1826.1										
					Total tannin extract						
39					21.3	39.6	2.1	52.2	6.0	62.5	37.5

Abbreviations: Epigall = epigallocatechin, Cat = Catechin, Ecat = Epicatechin, and Epicatgall = Epicatechin gallate. [a]Total tannin concentration at each DP following HPLC/phloroglucinolysis determined by the summation of individual subunit concentrations determined using conversion factors relative to catechin. [b]The proportion of the total tannin concentration for all DP calculated from the tannin concentration following HPLC/phloroglucinolysis and the calculation of individual subunits using conversion factors relative to catechin. [c]The proportion of the total concentration for all DP determined following HPLC/phloroglucinolysis and calculated as catechin equivalents. [d]The proportion of the total concentration for all DP determined by absorbance at 280 nm on a UV-Vis spectrophotometer before phloroglucinolysis.

Table 5.3. Distribution of tannin extracted from skin of Shiraz grapes harvested from high vigour canopy vines on Schwarzmann rootstock sourced from Sunraysia (Victoria, Australia).

	Total tannin concentration				% Conversion	% of extension subunits				% of terminal subunits	
DP	(mg/L HPLC)[a]	(% by HPLC)[b]	(% by HPLC CE)[c]	(% by UV-Vis CE)[d]	**yield**	Epigall	Cat	Ecat	Ecatgall	Cat	Ecat
4	45.0	2.0	2.2	10.2	6.2	11.6	1.8	79.6	7.0	81.8	18.2
5	35.0	1.6	1.7	5.8	8.5	12.8	1.8	78.1	7.4	83.7	16.3
6	43.2	2.0	2.1	5.0	12.3	12.3	1.4	79.0	7.2	84.4	15.6
7	29.9	1.4	1.5	2.5	17.2	12.6	1.3	78.9	7.2	83.6	16.4
8	93.4	4.2	4.6	7.5	18.6	13.7	1.9	76.6	7.8	81.1	18.9
9	44.0	2.0	2.2	3.4	19.8	12.2	1.5	79.0	7.3	81.8	18.2
10	45.1	2.0	2.2	2.8	24.5	12.3	1.4	78.9	7.3	83.0	17.0
12	122.6	5.5	5.9	5.3	31.2	20.0	0.9	71.2	8.0	87.1	12.9
15	277.7	12.6	13.2	15.3	24.2	26.4	1.1	62.8	9.7	86.9	13.1
26	270.4	12.2	12.1	13.4	23.7	31.8	1.0	59.0	8.3	89.4	10.6
35	225.7	10.2	9.7	8.5	30.1	33.5	1.0	58.6	6.8	87.3	12.7
39	240.6	10.9	10.0	5.6	35.8	36.3	1.0	56.0	6.7	84.5	15.5
41	385.5	17.4	15.9	7.7	36.0	35.8	1.1	57.0	6.1	82.3	17.7
42	221.8	10.0	11.3	4.8	37.7	35.4	1.2	57.9	5.5	77.1	22.9
44	130.0	5.9	5.3	2.2	40.4	35.5	1.2	57.8	5.5	77.6	22.4
Total (mg/L)	2209.9										
					Total tannin extract						
33					21.9	37.1	2.3	55.3	5.3	64.5	35.5

Abbreviations: Epigall = epigallocatechin, Cat = Catechin, Ecat = Epicatechin, and Epicatgall = Epicatechin gallate. [a]Total tannin concentration at each DP following HPLC/phloroglucinolysis determined by the summation of individual subunit concentrations determined using conversion factors relative to catechin. [b]The proportion of the total tannin concentration for all DP calculated from the tannin concentration following HPLC/phloroglucinolysis and the calculation of individual subunits using conversion factors relative to catechin. [c]The proportion of the total concentration for all DP determined following HPLC/phloroglucinolysis and calculated as catechin equivalents. [d]The proportion of the total concentration for all DP determined by absorbance at 280 nm on a UV-Vis spectrophotometer before phloroglucinolysis.

Table 5.4. Distribution of tannin extracted from skin of Shiraz grapes harvested from vines grown on Paulsen rootstock sourced from Sunraysia (Victoria, Australia).

	Total tannin concentration				% Conversion	% of extension subunits				% of terminal subunits	
DP	(mg/L HPLC)[a]	(% by HPLC)[b]	(% by HPLC CE)[c]	(% by UV-Vis CE)[d]	**yield**	Epigall	Cat	Ecat	Ecatgall	Cat	Ecat
3	23.9	1.1	1.2	6.1	5.7	11.7	2.7	78.9	6.6	77.6	22.4
4	30.5	1.4	1.5	4.8	9.2	11.7	1.8	79.7	6.9	81.7	18.3
5	40.1	1.8	2.0	4.4	12.4	12.7	1.5	78.7	7.0	85.5	14.5
6	57.3	2.6	2.9	5.5	16.4	12.6	1.6	78.2	7.6	87.2	12.8
7	49.5	2.2	2.5	4.2	16.7	12.2	1.5	78.5	7.8	85.2	14.8
8	119.6	5.4	6.0	8.8	19.0	12.7	1.5	78.2	7.7	79.6	20.4
10	27.2	1.2	1.4	1.2	29.7	13.8	0.8	77.8	7.6	85.0	15.0
13	196.7	8.9	9.6	6.2	41.1	21.8	0.9	69.0	8.2	90.8	9.2
14	247.3	11.2	11.7	17.1	18.9	26.3	1.4	63.7	8.6	91.7	8.3
24	306.8	13.9	13.6	13.1	28.6	34.8	1.4	56.0	7.8	87.8	12.2
32	262.7	11.9	11.3	8.4	37.2	35.0	0.9	57.9	6.1	82.9	17.1
38	221.2	10.0	9.5	5.9	44.3	34.6	0.8	58.8	5.8	83.8	16.2
41	408.1	18.5	17.6	9.5	51.2	33.9	0.8	60.0	5.3	71.9	28.1
42	118.5	5.4	5.0	2.6	52.4	33.8	0.9	60.1	5.2	70.8	29.2
45	97.8	4.4	4.1	2.1	54.4	34.0	0.9	60.0	5.1	68.6	31.4
Total (mg/L)	2207.3										
					Total tannin extract						
31					23.7	33.8	2.1	58.7	5.5	70.8	29.2

Abbreviations: Epigall = epigallocatechin, Cat = Catechin, Ecat = Epicatechin, and Epicatgall = Epicatechin gallate. [a]Total tannin concentration at each DP following HPLC/phloroglucinolysis determined by the summation of individual subunit concentrations determined using conversion factors relative to catechin. [b]The proportion of the total tannin concentration for all DP calculated from the tannin concentration following HPLC/phloroglucinolysis and the calculation of individual subunits using conversion factors relative to catechin. [c]The proportion of the total concentration for all DP determined following HPLC/phloroglucinolysis and calculated as catechin equivalents. [d]The proportion of the total concentration for all DP determined by absorbance at 280 nm on a UV-Vis spectrophotometer before phloroglucinolysis.

Table 5.5. Distribution of tannin extracted from skin of Shiraz grapes harvested from vines grown on own roots sourced from Sunraysia (Victoria, Australia).

	Total tannin concentration				% Conversion	% of extension subunits				% of terminal subunits	
DP	(mg/L HPLC)[a]	(% by HPLC)[b]	(% by HPLC CE)[c]	(% by UV-Vis CE)[d]	**yield**	Epigall	Cat	Ecat	Ecatgall	Cat	Ecat
4	29.9	1.4	1.5	5.7	7.5	14.1	2.0	77.2	6.6	84.8	15.2
5	35.5	1.6	1.8	5.4	9.3	15.8	1.4	75.5	7.3	85.3	14.7
6	28.3	1.3	1.5	3.5	11.7	13.4	1.3	78.3	7.0	85.8	14.2
7	133.6	6.1	6.9	12.1	16.0	14.0	1.5	76.5	8.0	86.3	13.7
8	48.6	2.2	2.5	3.8	18.4	14.6	1.5	75.9	8.0	83.8	16.2
9	71.5	3.3	3.7	6.3	16.6	15.0	1.5	75.5	8.1	82.8	17.2
10	9.7	0.4	0.5	1.0	13.9	14.5	1.4	76.3	7.8	77.1	22.9
12	35.1	1.6	1.8	1.5	32.9	15.9	1.0	75.4	7.6	88.5	11.5
14	167.1	7.6	8.4	6.7	34.7	20.8	0.7	71.0	7.4	90.6	9.4
16	292.8	13.4	13.8	16.3	23.6	31.8	1.6	58.1	8.6	87.2	12.8
28	294.2	13.4	13.0	11.0	32.9	36.8	1.1	55.2	6.8	81.2	18.8
37	449.5	20.5	19.4	12.4	43.9	36.1	0.9	57.7	5.3	74.3	25.7
39	91.2	4.2	3.7	1.9	53.3	35.7	0.9	58.6	4.8	60.3	39.7
45	257.7	11.8	11.1	6.4	48.9	35.6	0.8	58.7	4.8	70.8	29.2
46	245.1	11.2	10.5	6.0	48.5	35.3	0.9	59.0	4.8	72.4	27.6
Total (mg/L)	2189.8										
					Total tannin extract						
36					22.7	35.6	2.0	56.9	5.4	64.6	35.4

Abbreviations: Epigall = epigallocatechin, Cat = Catechin, Ecat = Epicatechin, and Epicatgall = Epicatechin gallate. [a]Total tannin concentration at each DP following HPLC/phloroglucinolysis determined by the summation of individual subunit concentrations determined using conversion factors relative to catechin. [b]The proportion of the total tannin concentration for all DP calculated from the tannin concentration following HPLC/phloroglucinolysis and the calculation of individual subunits using conversion factors relative to catechin. [c]The proportion of the total concentration for all DP determined following HPLC/phloroglucinolysis and calculated as catechin equivalents. [d]The proportion of the total concentration for all DP determined by absorbance at 280 nm on a UV-Vis spectrophotometer before phloroglucinolysis.

Table 5.6. Distribution of tannin extracted from skin of Shiraz grapes harvested from vines grown on Schwarzmann rootstock sourced from Glenrowan (Victoria, Australia).

	Total tannin concentration				% Conversion	% of extension subunits				% of terminal subunits	
DP	(mg/L HPLC)[a]	(% by HPLC)[b]	(% by HPLC CE)[c]	(% by UV-Vis CE)[d]	**yield**	Epigall	Cat	Ecat	Ecatgall	Cat	Ecat
4	27.1	1.6	1.7	10.5	3.5	16.1	2.2	75.3	6.4	79.6	20.4
5	27.1	1.6	1.8	6.6	5.7	16.1	2.1	74.7	7.1	82.0	18.0
6	22.0	1.3	1.5	4.1	7.4	17.5	1.9	72.5	8.1	81.4	18.6
7	54.4	3.2	3.6	7.7	10.0	17.2	2.0	71.9	8.9	79.1	20.9
8	66.5	3.9	4.4	7.0	13.3	18.3	2.0	70.9	8.8	76.2	23.8
9	48.8	2.9	3.1	4.7	14.1	22.9	2.2	66.5	8.4	70.3	29.7
12	14.0	0.8	0.9	1.0	19.0	23.3	1.5	67.3	7.9	75.3	24.7
15	110.3	6.4	7.1	5.9	24.9	26.5	1.3	63.1	9.1	90.9	9.1
20	259.7	15.2	16.2	13.5	24.9	32.3	1.5	56.5	9.7	91.0	9.0
33	248.9	14.5	14.5	12.7	23.7	38.4	1.7	51.6	8.3	87.0	13.0
47	283.3	16.6	15.5	9.2	43.9	42.1	2.0	49.5	6.4	76.8	23.2
48	281.2	16.4	15.3	9.7	32.8	42.0	1.9	49.7	6.4	81.5	18.5
50	108.9	6.4	5.9	3.2	38.8	41.9	1.9	49.9	6.3	79.7	20.3
51	159.0	9.3	8.5	4.3	41.6	42.6	2.1	49.2	6.1	74.2	25.8
Total (mg/L)	1711.4										
					Total tannin extract						
41					15.1	43.8	2.8	47.1	6.2	57.4	42.6

Abbreviations: Epigall = epigallocatechin, Cat = Catechin, Ecat = Epicatechin, and Epicatgall = Epicatechin gallate. [a]Total tannin concentration at each DP following HPLC/phloroglucinolysis determined by the summation of individual subunit concentrations determined using conversion factors relative to catechin. [b]The proportion of the total tannin concentration for all DP calculated from the tannin concentration following HPLC/phloroglucinolysis and the calculation of individual subunits using conversion factors relative to catechin. [c]The proportion of the total concentration for all DP determined following HPLC/phloroglucinolysis and calculated as catechin equivalents. [d]The proportion of the total concentration for all DP determined by absorbance at 280 nm on a UV-Vis spectrophotometer before phloroglucinolysis.

91 % while epicatechin ranged between 9 and 30 %.

Average DP and composition of the total extract

The total tannin extracts derived from Shiraz skin were analysed by phloroglucinolysis and HPLC to determine the average DP and composition prior to fractionation (Tables 5.1-5.6). The average DP for skin tannin extracted from grapes derived from low, medium and high vigour vines was 31, 39 and 33 respectively with percent conversion yields of 15, 21 and 22 %. Shiraz skin tannin extracted from grapes grown on Paulsen rootstock and own roots had an average DP of 31 and 36 respectively with percent conversion yields of 23 and 22 %. For Shiraz skin tannin extracted from grapes grown in Glenrowan, the average DP was 41 with a percent conversion yield of 15 %.

The extension subunit composition of the total tannin extracts was similar for grape skin from low, medium and high vigour vines with extension subunits epicatechin and epigallocatechin representing around 52 and 38 % respectively. The proportion of extension subunit epigallocatechin was slightly lower in skin tannin from Shiraz grapes grown on Paulsen and own roots representing around 35 % of all extension subunits while the proportion of epicatechin was slightly higher at around 57 %.

For skin tannin extracted from grapes sourced from Glenrowan, the proportion of extension subunit epigallocatechin was slightly higher than the proportion measured for grapes sourced from Sunraysia being 43 %, while the proportion of epicatechin was slightly lower at 47 %. The proportion of catechin and epicatechin gallate extension subunits represented around 2 and 5 % respectively for all Shiraz skin tannin extracts.

For terminal subunits, the proportion of catechin and epicatechin for all Shiraz skin extracts was similar, ranging between 56 and 71 % for catechin and 29 and 43 % for epicatechin.

Tannin binding capacity of cell walls

The tannin binding capacity of grape skin cell walls was determined by measuring tannin content by phloroglucinolysis and HPLC before and after the addition of a known amount of grape seed tannin to isolated cell wall material (Table 5.7).

Table 5.7. The tannin binding capacity of cell wall material isolated from skins of Shiraz grapes harvested on low, medium and high vigour vines grown on Schwarzmann rootstock or Paulsen rootstocks or on own roots in Sunraysia (Victoria, Australia) and vines grown on Schwarzmann rootstock in Glenrowan (Victoria, Australia).

	Amount of cell wall material (mg cell wall material/g skin)*	**Tannin binding capacity of cell wall (µg of tannin/mg of cell wall material)$^{+}$**
Low vigour vines	209 ± 36	3.9 ± 0.2
Medium vigour vines	218 ± 20	3.7 ± 0.2
High vigour vines	352 ± 18	3.4 ± 0.1
Paulsen rootstock	360 ± 29	4.0 ± 0.3
Own roots	300 ± 22	5.0 ± 0.3
Glenrowan	303 ± 51	4.1 ± 0.4

*Values are means of two replicates ± standard error $^{+}$ Values are means of three replicates ± standard error

The tannin binding capacity ranged between 3.4 and 5.0 µg of tannin per mg of Shiraz grape skin cell wall material. The binding capacity of cell wall material isolated from the skin of grapes grown on low, medium and high vigour vines decreased slightly from 3.9 to 3.4 µg/mg of cell wall material with less tannin bound to cell wall material isolated from vines with greater vigour. Skin cell wall material from grapes grown on Paulsen rootstock had a similar binding capacity to the cell walls isolated from the

grapes of low vigour vines grown on Schwarzmann rootstock. The cell wall material isolated from skins of Shiraz grapes grown on own roots had the highest tannin binding capacity being 5.0 μg/mg of cell wall material.

The tannin binding capacity of skin cell wall material from Shiraz grown on Schwarzmann rootstock in Glenrowan was similar to low vigour vines grown on Schwarzmann and Shiraz grown on Paulsen from Sunraysia, binding 4.1 μg/mg of cell wall material.

The amount of cell wall material isolated from Shiraz grape skin was also determined (Table 5.7). For low, medium and high vigour canopy vines, the amount of skin cell wall material increased with increasing vigour from 209 to 352 mg/g of skin. The amount of skin cell wall material was highest for Shiraz grown on Paulsen rootstock, while Shiraz grown on own roots and from Glenrowan grown on Schwarzmann rootstock, had a similar amount of cell wall material being around 300 mg/g of skin.

Winemaking

Tannin extraction

The amount of tannin extracted from grapes during winemaking was determined by analysing the amount of tannin present in grape skin, seeds and wine (Table 5.8).

Shiraz grapes grown on Paulsen rootstock contained the highest amount of skin tannin at 5.5 mg/g fresh weight of skin, while Shiraz grapes grown on Schwarzmann rootstock in Glenrowan had the highest amount of seed tannin at 23.1 mg/g of seed. There was a slight increase in skin tannin from 3.0 mg/g of tannin in the skin of low vigour vines to 4.4 mg/g in high vigour vines.

When total tannin (skin and seed combined) was considered, Shiraz grown on Paulsen rootstock in Sunraysia and Shiraz grown on Schwarzmann rootstock in Glenrowan

yielded the highest tannin levels both being 27.0 mg/g of seed and skin. Low vigour vines grown on Schwarzmann rootstock gave the lowest total tannin levels at 22.4 mg/g of seed and skin.

The concentration of wine tannin was lowest for Shiraz from Sunraysia grown on own roots at 118.0 mg/L and highest for Shiraz grown on Schwarzmann rootstock in Glenrowan at 360.2 mg/L. The tannin content of wine made from Glenrowan Shiraz grapes was much higher than wines made from Sunraysia fruit. The concentration of tannin in wine from Shiraz grown on Paulsen rootstock was 183.0 mg/L. The amount of tannin extracted into wine from grapes grown on low, medium and high vigour vines grown on Schwarzmann rootstock in Sunraysia increased as canopy vigour decreased from 148.7 mg/L for wine derived from high vigour vines to 243.9 mg/L for wines derived from low vigour vines.

The degree of polymerisation (DP) of wine tannins ranged from 4.9 to 8.5 subunits. For wines made from low, medium and high vigour vines grown on Schwarzmann rootstock, there was a slight decrease in wine DP with increasing canopy vigour from a DP of 8.5 for wines derived from low vigour vines to 5.6 for wines derived from high vigour vines. For wine made from Shiraz vines grown on Paulsen rootstock and own roots, DP was 6.8 and 4.9 respectively, while wine made from Shiraz grown on Schwarzmann rootstock in Glenrowan had a DP of 6.5.

A slight decrease in the proportion of the extension subunit epigallocatechin was observed for wines made from vines with increasing canopy vigour from 24.2 % of extension subunits for wines corresponding to low vigour vines to 20.8 % for wines corresponding to high vigour vines. The proportion of extension subunit epicatechin increased slightly with increasing vigour from 69.9 % for wines from fruit of low vigour vines to 73.0 % for wines from fruit of high vigour vines.

The subunit composition of wine made from Shiraz grapes grown on Paulsen rootstock and own roots in Sunraysia and on Schwarzmann rootstock in Glenrowan were all similar. For these wines, the proportion of the extension subunit epigallocatechin ranged from 17 to 20 % of extension subunits, while epicatechin ranged from 71 to 77 %.

The proportion of catechin and epicatechin gallate extension subunits were similar in all wines with catechin representing between 1 and 3 % and epicatechin gallate representing between 3 and 8 % of all extension subunits.

The proportion of terminal subunits was also similar for all wines with catechin most abundant representing between 54 and 61 %, epicatechin represented between 30 and 35 % and epicatechin gallate was least abundant at between 7 and 10 %.

Table 5.8. Composition of tannin in grape skin, seeds and wine derived from grapes grown under a range of environmental conditions*. Mean values within each row with the same letter(s) are not significantly different at $p<0.05$.

	Low vigour vines	Medium vigour vines	High vigour vines	Paulsen rootstock	Own roots	Glenrowan
Total skin tannin (mg/g skin)	3.0 ± 0.1^{a}	4.2 ± 0.2^{b}	4.4 ± 0.06^{b}	5.5 ± 0.4^{c}	4.7 ± 0.1^{b}	4.2 ± 0.2^{b}
Total seed tannin (mg/g seed)	19.4 ± 0.6	19.8 ± 0.2	21.1 ± 0.2	21.6 ± 1.3	21.3 ± 1.4	23.1 ± 1.6
Total skin and seed tannin (mg/g)	22.4 ± 0.7^{a}	24.0 ± 0.4^{ab}	25.5 ± 0.3^{bc}	27.1 ± 1.7^{c}	26.0 ± 1.5^{bc}	27.3 ± 1.8^{c}
Total wine tannin (mg/L)	243 ± 14^{c}	229 ± 13^{c}	148 ± 10^{ab}	183 ± 7^{b}	118 ± 15^{a}	360 ± 22^{d}
Wine Tannin Composition						
DP	8.5 ± 0.3^{d}	6.9 ± 0.1^{c}	5.6 ± 0.3^{b}	6.8 ± 0.2^{c}	4.9 ± 0.3^{a}	6.5 ± 0.1^{c}
% Epigallocatechin extension	24.2 ± 0.2^{c}	21.0 ± 0.6^{b}	20.8 ± 0.1^{b}	19.7 ± 0.8^{ab}	17.6 ± 1.5^{a}	19.7 ± 0.3^{ab}
% Catechin extension	1.3 ± 0.09^{a}	1.1 ± 0.06^{a}	1.4 ± 0.05^{a}	1.1 ± 0.02^{a}	2.2 ± 0.6^{b}	1.3 ± 0.08^{a}
% Epicatechin extension	69.9 ± 0.2^{a}	72.3 ± 0.5^{b}	73.0 ± 0.2^{b}	73.8 ± 0.6^{b}	77.3 ± 1.6^{c}	71.7 ± 0.2^{ab}
% Epicatechin gallate extension	4.7 ± 0.05^{b}	5.6 ± 0.1^{c}	4.8 ± 0.2^{bc}	5.4 ± 0.2^{bc}	2.8 ± 0.6^{a}	7.3 ± 0.2^{d}
% Catechin terminal	57.2 ± 2.0	55.6 ± 2.1	52.0 ± 2.8	59.5 ± 1.7	60.6 ± 1.8	54.8 ± 1.2
% Epicatechin terminal	33.7 ± 1.8^{a}	37.7 ± 1.7^{b}	38.0 ± 2.2^{b}	33.0 ± 1.4^{b}	29.9 ± 1.5^{a}	34.9 ± 1.3^{ab}
% Epicatechin gallate terminal	9.1 ± 0.2^{b}	6.6 ± 0.4^{ab}	10.0 ± 0.6^{b}	7.5 ± 0.4^{b}	9.4 ± 0.7^{b}	10.3 ± 0.4^{b}

*Values are means of three replicates ± standard error

Anthocyanin extraction

The amount of anthocyanin determined in grape skin and wine is shown in Table 5.9. For grape skin from Sunraysia, similar amounts of anthocyanin were found ranging between 2.5 and 3.2 mg/g of skin. The anthocyanin concentration of grapes grown in Glenrowan was substantially higher at 5.5 mg/g of skin.

In wine, the concentration of anthocyanin decreased with increasing canopy vigour. For wines made from grapes grown on Schwarzmann rootstock with low vigour vines, the concentration of anthocyanin was 4.0 mg/mL decreasing to 2.0 mg/mL for wines corresponding to high vigour vines. Wines made from grapes grown on Paulsen rootstock and own roots contained the lowest wine anthocyanin concentrations at 1.9 and 1.6 mg/mL respectively, while wines made from grapes grown on Schwarzmann rootstock in Glenrowan had the highest wine anthocyanin concentration at 6.3 mg/mL.

Colour and co-pigmentation of small scale wines

Colour and co-pigmentation parameters for small scale wines are shown in Table 5.9 at the end of fermentation. Wine made from grapes grown on Schwarzmann in Glenrowan yielded the highest wine colour parameters with a wine colour density of 19 au, total anthocyanins of 665.2 mg/mL, total red pigments of 42.4 au and total phenolics of 54.2 au.

Wines made from fruit grown in Sunraysia had much lower wine colour than wine from Glenrowan. Grapes grown on Paulsen rootstock and own roots produced wine with the lowest colour density of 3.4 and 3.6 au respectively. Total anthocyanins were 225 mg/mL for wines made from grapes grown on Paulsen rootstock and 183.1

Table 5.9. Anthocyanin content in grape skin and wine, wine colour and co-pigmentation parameters for small scale wines at the end of fermentation *. Mean values within each row with the same letter(s) are not significantly different at $p<0.05$.

	Low vigour vines	Medium vigour vines	High vigour vines	Paulsen rootstock	Own roots	Glenrowan
			Anthocyanins			
Total skin anthocyanin (mg/g skin)	2.5 ± 0.06^{a}	2.8 ± 0.07^{b}	2.7 ± 0.03^{ab}	3.2 ± 0.05^{c}	2.5 ± 0.09^{ab}	5.5 ± 0.15^{d}
Total wine anthocyanin (mg/mL)	4.0 ± 0.03^{d}	3.7 ± 0.1^{c}	2.1 ± 0.02^{b}	1.9 ± 0.05^{b}	1.6 ± 0.05^{a}	6.3 ± 0.05^{e}
			Wine colour			
Wine colour density (au)	10.9 ± 0.3^{c}	8.9 ± 0.5^{b}	3.6 ± 0.1^{a}	3.4 ± 0.1^{a}	3.6 ± 0.2^{a}	19 ± 0.7^{d}
Wine colour hue (au)	0.5 ± 0.01^{a}	0.5 ± 0.002^{a}	0.6 ± 0.02^{bc}	0.6 ± 0.01^{b}	0.6 ± 0.01^{c}	0.8 ± 0.02^{d}
Total anthocyanins (mg/L)	529.5 ± 6.8^{e}	451.1 ± 6.0^{d}	272.9 ± 5.7^{c}	225.0 ± 7.3^{b}	183.1 ± 6.1^{a}	665.2 ± 21.5^{f}
Ionised anthocyanins (mg/L)	90.9 ± 6.1^{bc}	77.5 ± 5.5^{b}	26.7 ± 2.0^{a}	25.4 ± 2.5^{a}	22.9 ± 1.6^{a}	100.7 ± 6.9^{c}
Total red pigments (au)	30.9 ± 0.3^{e}	25.9 ± 0.2^{d}	15.3 ± 0.3^{c}	12.8 ± 0.3^{b}	11.0 ± 0.3^{a}	42.4 ± 0.8^{f}
Total phenolics (au)	36.2 ± 0.7^{d}	30.5 ± 0.6^{c}	16.9 ± 0.2^{b}	14.0 ± 0.6^{a}	13.6 ± 0.4^{a}	54.2 ± 1.5^{e}
			Co-pigmentation			
% Colour due to anthocyanins	34.1 ± 1.0^{cd}	25.3 ± 1.9^{a}	32.0 ± 2.2^{bc}	28.1 ± 0.9^{ab}	25.2 ± 0.4^{a}	38.1 ± 1.0^{d}
% Colour due to co-pigmentation complex	35.8 ± 2.2^{b}	48.4 ± 3.1^{cd}	44.5 ± 2.7^{c}	53.0 ± 1.8^{d}	48.7 ± 0.8^{cd}	10.3 ± 1.7^{a}
% Colour due to polymeric pigments	30.1 ± 1.3^{c}	26.3 ± 1.2^{b}	23.5 ± 0.8^{b}	18.9 ± 1.0^{a}	26.1 ± 0.8^{b}	51.6 ± 1.1^{d}

*Values are means of three replicates ± standard error

mg/mL for grapes grown on own roots. Total red pigments and total phenolics were also low being 12.8 au red pigments for wines made from grapes grown on Paulsen rootstock and 11 au for wines made from grapes grown on own roots. Total phenolics for grapes grown on Paulsen rootstock and own roots were 14 and 13.6 au respectively. Wine colour parameters decreased in wines made from grapes grown on Schwarzmann rootstock with increasing vine vigour. Wine colour density decreased from 10.9 to 3.6 au for wines corresponding to low and high vigour vines while total anthocyanin levels decreased from 529.5 to 272.9 mg/mL and total red pigments decreased from 30.9 to 15.3 au with decreasing vine vigour.

In terms of co-pigmentation, wines made from grapes grown on Schwarzmann in Glenrowan had the highest percent of colour due to anthocyanins and polymeric pigments representing 38.1 and 51.6 % respectively, but the lowest percent of colour due to co-pigmentation complex representing 10.3 %.

The percent of colour due to polymeric pigments was much lower in wines made from grapes grown in Sunraysia compared to wines made from grapes grown in Glenrowan. Wines made from grapes grown on Paulsen in Sunraysia had the highest percent of colour due to co-pigmentation (53 %) and the lowest percent of colour due to polymeric pigments (18.9 %). There were no other obvious trends for wine co-pigmentation parameters except for a decrease in the percent of colour due to polymeric pigments in wines made from grapes grown with increasing vine canopy vigour from 30.1 % polymeric pigments in wines made from low vigour vines to 23.5 % in wines made from high vigour vines.

DISCUSSION

Skin tannin DP range and distribution

The aim of this chapter was to explore the potential variability of tannin distribution by analysing grapes grown across a range of environmental conditions. To achieve this, the tannin content of skin of Shiraz grapes grown in Sunraysia on Schwarzmann rootstock with increasing canopy vigour, on Paulsen rootstock and own roots grown in Sunraysia as well as on Schwarzmann rootstock from the cooler growing region of Glenrowan was analysed and evaluated.

The DP for the different Shiraz skin samples ranged from 3 to 51 subunits. Shiraz skin from grapes grown in Glenrowan gave the largest DP range being 4 to 51 subunits, while Shiraz skin from high vigour vines had the smallest DP range with DP reported from 4 to 44 subunits. In Chapter 2, the DP range of Shiraz grown in the 2009 season ranged from 4 to 65 subunits, which was a much larger range than that observed for samples sourced in the 2010 season. Prior to this study, the DP range of Shiraz skin had not been reported. In Chapter 2, DP range of Cabernet Sauvignon skin from the

2009 season was reported to be similar to earlier studies (*94, 95*) suggesting that DP range might be influenced by variety. However, the difference in DP range observed in Shiraz between the 2009 and 2010 season would suggest that DP range can also be influenced by seasonal variation. The DP range of Shiraz grown under different environmental conditions in the 2010 season showed less variability than the compositional differences observed between Shiraz grapes grown in 2009 and 2010.

For grapes grown in the 2010 season, the Shiraz grown in Glenrowan gave a larger DP range than all the Shiraz grapes grown in Sunraysia, which may suggest that the factors between different regions that influence variability are most likely those that can be associated with seasonal differences such as temperature rather than factors relating to vineyard site such as soil and topography.

Generally, the distribution patterns of tannin concentration at different DP for all Shiraz skin tannin samples were similar to those reported in Chapter 2, with low tannin concentration at small DP and higher tannin concentration at high DP. However, a comparison of DP with the highest tannin concentration measured by HPLC showed some variation in the Shiraz skin tannin distribution between samples in the 2010 season. For Shiraz grown in Glenrowan, and for low and medium vigour Shiraz vines, the DP present in the largest proportion when calculated by HPLC was DP 20. Although, for Shiraz grown on Paulsen and own roots and for high vigour Shiraz vines, the DP present in the largest proportion (calculated by HPLC), was substantially higher at DP 41, 37 and 41 respectively. Interestingly, when the concentration of tannin was measured in wines made from these grapes, the wines with the lowest tannin concentrations were from the Paulsen, own roots and high vigour vines. In Chapter 2, it was concluded that tannin with a DP higher than 20 was not extracted into wine. The work reported in this chapter supports this conclusion as

wines with the highest tannin concentration were made from fruit that had a higher proportion of tannin with DP 20.

Skin tannin composition

In addition to tannin concentration at each DP, the subunit composition was also determined at each DP for all Shiraz skin tannin samples from the 2010 season.

For all samples, the proportion of epigallocatechin was found to increase with increasing DP, as reported in Chapter 2 and in earlier studies (*91, 95, 155*). In the 2010 season, Shiraz grown in Glenrowan had the largest DP range and at all DP also had a slightly higher proportion of epigallocatechin than Shiraz grapes grown in Sunraysia. This might be related to the relationship between the increasing proportion of epigallocatechin with increasing DP, which suggests that grapes with a larger DP range will have a higher proportion of epigallocatechin, but may also be driven by other factors. This was also observed in the distribution of tannin in Cabernet Sauvignon skin reported in Chapter 2, which had a larger DP range and higher proportion of epigallocatechin than was observed for Shiraz.

While it is doubtful that the proportion of epigallocatechin is pre-determined by a given DP, these results suggest that viticultural management techniques which increase the DP range, might also increase the proportion of epigallocatechin in the grape skin.

A decrease in the proportion of epigallocatechin has previously been reported with increasing canopy vigour and shading (*92, 145*). In the present study, a small decrease in the proportion of epigallocatechin was observed with increasing canopy vigour in the distribution range of tannin with a DP less than 20.

A small difference was also seen in the composition of skin tannin for Shiraz grapes grown on Paulsen rootstock and own roots. The tannin composition of Shiraz grapes

grown on Paulsen rootstock was more similar to the grapes grown on Schwarzmann with a high vigour canopy while Shiraz grown on own roots was most similar to the composition of skin tannin from the medium and low canopy vigour vines. It remains unclear what factors may determine these small differences in composition, but it is likely the effect that different rootstocks have on canopy vigour or the mix of clones for Shiraz vines grown on own roots influenced both tannin composition and DP range.

It has previously been hypothesised that grapes grown in cooler climates have lower proportions of epigallocatechin (*89*), but that was not observed in the current study. The cooler growing region of Glenrowan yielded grapes with a higher proportion of epigallocatechin than the grapes grown in the warm climate of Sunraysia. While temperature is a critical environmental variable for grapes grown in different regions, it could be the effect of bunch exposure and canopy vigour that determined the difference in composition in this study. Grapes grown in Glenrowan had comparatively lower canopy vigour with greater bunch exposure compared to grapes grown in Sunraysia, which were encouraged to develop large canopies in order to protect grape bunches from sunburn. The shading level of different canopies can be pre-determined according to the viticultural management practices employed in cooler and warm climate regions and could be a critical factor in determining differences in tannin distribution observed in this study.

While some variation was observed in tannin distribution of Shiraz grown in the 2010 season, the seasonal impacts on composition were greater than vigour, rootstock or region. In the 2010 season, the proportion of epigallocatechin was much lower in all Shiraz samples than that reported for Shiraz skin tannin samples obtained in the 2009 season. The higher proportion of epigallocatechin in the 2009 season was also

accompanied by a larger DP range, which had been observed in the tannin distribution of Cabernet Sauvignon from Sunraysia and Shiraz from Glenrowan.

Wine tannin composition

Wine tannin composition was similar to fruit tannin composition below DP 20. The average DP in wine was similar to that reported in Shiraz and Cabernet Sauvignon wines in Chapter 2 further suggesting that DP above 20 in grapes is not extracted into wine as it most likely remains bound to the cell wall. The proportion of epigallocatechin in wine was most similar to the proportion of epigallocatechin reported in grape skin tannin below DP 16. The small decrease in the proportion of epigallocatechin reported in grape skin tannin with increasing canopy vigour was also observed in wine.

However, the higher proportion of epigallocatechin observed in grapes grown in Glenrowan at a DP below 20, was not observed in the wine. One explanation for this could be that tannin with a higher proportion of epigallocatechin is favoured in the formation of polymeric pigments and therefore not measured by phloroglucinolysis (*156, 157*). Indeed, wine made from grapes grown in Glenrowan had a higher proportion of anthocyanin and polymeric pigments. The preference for epigallocatechin to form polymeric pigments is also consistent with the decrease in the proportion of epigallocatechin observed with decreasing DP and percent conversion yield. Lower percent conversion yields have been attributed to higher proportions of polymeric pigments that are not detectable by phloroglucinolysis (*156, 157*). The preference for tannins composed of epigallocatechin to form polymeric pigments remains to be established as any previous studies that investigate the formation of polymeric pigments have focused on tannins composed of epicatechin and catechin rather than epigallocatechin (*61, 158*).

This study demonstrates the benefit of determining the distribution of grape skin tannin when predicting wine tannin composition. An average measure of grape skin tannin provides an over estimation of potential wine tannin, since larger tannins (i.e. DP>20) are not extracted into wine. Consideration of tannin distribution provides information enabling assessment of the range of DP that is most likely to be extracted into wine.

Wine tannin extraction

The amount of tannin extracted from grapes into wine was determined to investigate the influence of environmental factors on the tannin binding capacity of Shiraz skin cell walls. The concentration of tannin in wines was not correlated with the amount of tannin measured in fruit, as previously reported (*32, 90*). While grapes grown in Glenrowan on Schwarzmann rootstock and in Sunraysia on Paulsen rootstock had the highest amount of tannin in fruit, wine made from Glenrowan Shiraz had a considerably higher tannin concentration than other wines, while wine made from grapes grown on Paulsen rootstock had a much lower tannin concentration. For grapes grown on Schwarzmann rootstock with low, medium and high vigour vines, the amount of tannin in the fruit was lowest for low vigour vines and highest in high vigour vines, but was the opposite for wine; i.e. low vigour vines had the lowest fruit tannin content (total skin and seed tannin), but the highest wine tannin concentration, while high vigour vines had the highest fruit tannin content (total skin and seed tannin) and the lowest wine tannin concentration.

For grapes grown in Sunraysia, there was a relationship between the amount of tannin extracted into wine and the amount of skin cell wall material. Higher amounts of skin cell wall material correlated with a lower tannin concentration in wine. This supports the conclusion drawn in Chapter 4 that the amount of cell wall material might play a

role in determining the amount of tannin extracted into wine. For Shiraz grapes harvested from low, medium and high canopy vigour vines, the amount of cell wall material increased with increasing vigour. The increasing amount of cell wall material correlated with a decrease in the amount of tannin extracted into wine. Shiraz grapes grown on Paulsen rootstock and on own roots also had higher quantities of cell wall material, but relatively low concentrations of tannin in wine.

The relationship between higher amounts of cell wall material and decreased amounts of tannin extracted into wine supports the hypothesis that cell walls influence tannin extraction into wine. One explanation for a decrease in tannin extraction with higher amounts of cell wall material is that cell walls might be thicker and more dense, which increases the amount of time it takes for tannin to diffuse through the cell wall into wine (*46*). The density and porosity of cell walls may also prevent the diffusion of tannins larger than DP 20 from moving through the cell wall, which could explain why tannin with a DP larger than 20 was not readily extracted into wine. This suggests that the extraction of tannin from cell walls is likely to be influenced more by the encapsulation and entrapment of tannin within the cell wall during berry development than binding interactions of tannins to cell walls of different composition or different tannin binding capacity during winemaking. In this study, no relationship was observed between the amount of tannin extracted into wine, differences in tannin composition, cell wall composition or the tannin binding capacity of cell walls.

While there was a relationship between the amount of cell wall material and the amount of tannin extracted into wine for Shiraz grapes grown in Sunraysia, the relationship did not apply to Shiraz grapes grown in Glenrowan. The amount of cell wall material in Shiraz skin cell walls from fruit grown in Glenrowan was similar to grapes grown in Sunraysia, but the amount of tannin extracted into wine was much

higher in wine made from grapes grown in Glenrowan. The tannin binding capacity of cell walls was also similar for grapes grown in Glenrowan and Sunraysia.

It is most likely that the difference in the amount of tannin extracted into wine between Sunraysia and Glenrowan fruit was influenced by other grape compositional factors. In Chapter 3, it was hypothesised that the ratio of anthocyanin to tannin may influence tannin stability and wine aging. In grapes grown in Glenrowan, the concentration of anthocyanin was much higher in both grapes and wine. It is thought that the higher concentration of anthocyanin in grapes grown in Glenrowan might have stabilised tannin in solution during fermentation through formation of more stable polymeric pigments. This is supported by the much higher proportion of polymeric pigments formed in wine made from grapes grown in Glenrowan compared to wines made from grapes grown in Sunraysia.

It is likely both the amount of cell wall material and the ratio of anthocyanin to tannin play a role in determining the tannin concentration in final wine. The amount of tannin extracted during winemaking is first determined by the amount of skin cell wall material, which is a physical barrier to tannin extraction. This may indicate the extent to which tannin is entrapped within the cell wall and its rate of diffusion out of the cell wall. Once extracted from the cell wall into the fermentation matrix, the stability of tannin is then influenced by the ratio of anthocyanin to tannin, with higher amounts of anthocyanin increasing the stability of tannin in wine.

CONCLUSIONS

The distribution of tannin in the skin of Shiraz grapes grown under a range of environmental conditions during the 2010 season showed some variation. The DP range was largest for grapes grown in the cooler growing region of Glenrowan. The

proportion of epigallocatechin decreased slightly with increasing canopy vigour and was highest in grapes grown in Glenrowan.

Determining the distribution of skin tannin in terms of composition and concentration at a range of DP provided a more accurate estimate of the composition of tannin extracted into wine. Tannin with a DP above 20 was not extracted into wine, whereas skins with a higher concentration of tannin at DP 20 yielded wines with higher tannin concentrations.

There was no overall relationship between the tannin binding capacity of skin cell walls and the amount of tannin extracted from grapes into wine. However, for grapes grown in Sunraysia, the amount of skin cell wall material correlated with the amount of tannin extracted into wine. Higher amounts of cell wall material correlated with less tannin extracted into the wine. More tannin was extracted from grapes harvested from the cooler growing region of Glenrowan despite similar levels of tannin and cell wall material being measured in the fruit. This could be attributable to the higher anthocyanin content of Glenrowan grapes, which might therefore better stabilise tannin during extraction.

CHAPTER 6. SUMMARY AND FUTURE DIRECTIONS

It is thought that tannins bind to cell walls preventing their extraction from grapes into wine and differences in tannin and polysaccharide composition will influence the strength of these interactions and the tannin binding capacity of cell walls.

This study aimed to investigate the tannin and polysaccharide composition of wine grapes in Shiraz and Cabernet Sauvignon and the relationship between tannin composition, polymer length, polysaccharide composition, the tannin binding capacity of grape cell walls and the amount and type of tannin extracted into wine.

The influence of environmental factors such as region and viticultural management were also investigated to determine the extent of variation in tannin and cell wall composition in Shiraz wine grapes and the influence of that variability on tannin extraction.

TANNIN DISTRIBUTION IN WINE GRAPES

Determining tannin distribution in grape skin, seed and wine in terms of the distribution of tannin polymer length, tannin concentration and subunit composition provided a detailed characterisation of condensed tannins in grapes and wine. While grape seed tannin distribution was similar between the varieties studied, skin tannin distribution was influenced by variety and environmental factors such as season and vine canopy vigour at commercial harvest. Cabernet Sauvignon skin tannin comprised a larger DP range and higher proportion of the subunit epigallocatechin than Shiraz skin tannin. A difference in the DP range and proportion of epigallocatechin in Shiraz

skin tannin was observed between growing seasons, but these parameters were also influenced by other environmental factors such as region and canopy vigour. The DP range and proportion of epigallocatechin in Shiraz skin tannin has higher for the cooler growing region and increased with decreasing canopy vigour.

The DP range and composition of tannin extracted from grapes into wine was similar to the distribution of grape skin tannin with a DP less than 20. It is most likely that tannin polymers above DP 20 are not extracted from grapes into wine, but instead remain entrapped within the cell wall. Considering that tannins with a DP above 20 appear not to be extracted into wine, further research on grape tannin distribution in relation to wine quality should probably focus on grape tannin with a DP below 20. A more thorough characterisation and understanding of the variation and structure of individual tannin polymers below DP 20 and their contribution to wine sensory character and long term wine colour stability would help to elucidate which tannins are most important to wine quality.

CELL WALL COMPOSITION IN WINE GRAPES

To investigate the influence of cell wall composition on the amount of tannin extracted from grapes into wine, the polysaccharide composition, the tannin binding capacity and the amount of cell wall material was determined in grape skin and in whole berries (whole berries with the seeds removed). Skin cell wall composition was investigated to determine the influence of tannin-cell wall interactions during the extraction of tannin from skin cell walls into the fermentation matrix, while the cell wall composition of whole berries (with seeds removed) was investigated to determine the extent to which polysaccharides derived from both skin and flesh that are present in the fermentation matrix, contribute to tannin-cell wall binding.

The polysaccharide composition of grape skin and whole berries was different. Compositional differences were also observed between Shiraz and Cabernet Sauvignon berries. There was variation in polysaccharide composition with maturity for both varieties, but no consistent trend was apparent. While the tannin binding capacity of Shiraz and Cabernet Sauvignon skin cell walls were similar, a decrease in the tannin binding capacity of Cabernet Sauvignon whole berries was observed with maturity. However, there was no link between polysaccharide composition and the tannin binding capacity of the cell walls. That is, determining the polysaccharide composition or tannin binding capacity of grapes did not indicate the amount of tannin that might be extracted into wine.

The amount of skin cell wall material varied between Shiraz and Cabernet Sauvignon and in Shiraz grown under different environmental conditions. There was a relationship between the amount of skin cell wall material and the rate of tannin extraction during fermentation in Shiraz and Cabernet Sauvignon and the amount of tannin extracted from grapes into wine in Shiraz grown under a range of environmental conditions. Less tannin was extracted into wine with grapes that had higher amounts of skin cell wall material. This could be related to the thickness and density of cell walls, which may influence the rate of tannin diffusion from the cell walls during tannin extraction. This suggests that the amount of tannin extracted into wine is more strongly influenced by the extent to which tannins are entrapped within the skin cell wall rather than the strength of binding between tannins and polysaccharides of different composition. Further research on the influence of cell walls on tannin extraction should probably focus on investigating structural features of skin cell walls that influence the rate of diffusion through the cell wall, such as cell wall thickness, density and porosity.

WINE TANNIN EXTRACTION

The hypotheses for this research were that 1) the binding of tannins to cell walls prevents extraction from grapes into wine and that 2) differences in tannin and polysaccharide composition will influence the strength of these interactions and therefore the tannin binding capacity of cell walls.

Results from this study support the hypothesis that cell wall interactions prevent tannin extraction from grapes into wine. There was a relationship between the amount of cell wall material and the amount of tannin extracted into wine. However, tannin and polysaccharide composition did not play a significant role in determining the strength of tannin and cell wall interactions. Structural features of cell walls such as the entrapment of tannin within the cell wall during berry development and the porosity of cell walls that prevent the diffusion of tannin with a DP greater than 20 may play a more significant role in determining the amount of tannin extracted into wine.

The extraction of tannin from grapes into wine is most likely dependent on the extent to which cell walls can be broken down during fermentation to allow release of tannins from the cell wall. The use of enzymes that break down the cell wall during winemaking would likely improve tannin extraction by releasing tannins entrapped within the cell wall.

This study also found that anthocyanin may play a significant role in determining the stability of tannin in wine following tannin extraction from the cell wall. While it has previously been hypothesised that anthocyanin is required to stabilise tannin in wine (*130*), there are few studies that investigate the influence of the anthocyanin to tannin ratio on the solubility and stability or amount of tannin extracted into wine (*129*). This is an area that would benefit from further research.

CONCLUSIONS AND FUTURE DIRECTIONS

This study provides new knowledge concerning tannin distribution in wine grapes and the type of tannin extracted into wine. It was thought that both tannin and cell wall composition influenced tannin extraction. The research presented here showed that while both tannin and polysaccharide composition vary in wine grapes, other factors such as the amount of cell wall material and ratio of anthocyanin to tannin also influence tannin extraction during winemaking.

One key conclusion from this research was that the thickness and density of the cell wall and the entrapment of tannins within the cell wall play a more significant role than binding interactions of tannins and polysaccharides for determining the amount of tannin extracted into wine. This conclusion raises a number of issues that could be addressed in further research in order to better understand the factors that influence tannin extraction. These include:

- The structural characteristics of cell walls that influence the quality and rate of tannin extraction
- The environmental factors and the stages of berry development and ripening which influence these structural characteristics
- The stages of berry development and during which tannin entrapment within the cell wall occurs
- Confirmation that tannins with a DP larger than 20 are entrapped within the cell wall preventing their extraction

Another conclusion from this research was that tannins above a DP of 20 were not extracted into wine as they may be bound or entrapped within the cell wall. If only tannin below a DP of 20 is extracted into wine, then future research on structurally characterising tannins should focus on tannin in this DP range. The knowledge gained

from accurately determining the structures of tannin that are extracted into wine would increase our understanding of which tannins are important for astringency and wine colour stability.

Research is also needed to determine the role of anthocyanins in tannin stability in wine. The work presented here suggests that anthocyanin may influence the amount of tannin extracted into wine and therefore tannin stability as wine ages. Future research should focus on investigating the solubility and stability of tannin in the presence of anthocyanin and its involvement in tannin extraction. To date, research on the interactions of tannins and anthocyanins have focused on wine aging rather than any interactions that might occur during fermentation and extraction.

In summary, the research presented here supports the hypothesis that cell walls influence the amount of tannin extracted into wine. However, other factors such as the ratio of anthocyanin to tannin may also play a role in determining the stability of tannin extracted into wine. Further research investigating cell wall structure and the ratio of anthocyanin to tannin in wine grapes would provide important knowledge that could be used to develop a predictive model for determining tannin extraction from grapes into wine.

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APPENDIX 1.

SUPPORTING INFORMATION CHAPTER 2

Supporting Information

Table 1. Shiraz seed PA separated by semi-preparative diol phase chromatography to report DP, PA concentration, percent conversion yield and the proportion of extension and terminal subunits in individual fractions. Fraction number corresponds to the time of elution (minutes). PA was not detected in seed fractions prior to Fraction 17.

Shiraz seed											
Fraction #	**DP**	**PA Concentration**			**% Conversion Yield**	**% of Extension subunits**			**% of Terminal subunits**		
		(mg/L HPLC)[a]	(mg/L HPLC CE)[b]	(mg/L UV-Vis CE)[c]		Cat	Ecat	Ecatgall	Cat	Ecat	Ecatgall
17	1.6±0.1	28.1±1.5	28.1±1.2	71.1±12.4	42.8±9.5	5.0±0.8	95.0±0.8	n.d.	18.4±1.4	40.7±2.0	40.8±3.4
18	2.1±0.04	36.6±1.4	36.6±1.3	72.6±7.0	51.6±6.2	5.2±0.5	94.8±0.5	n.d.	33.6±0.9	63.1±0.4	3.2±1.0
19	2.1±0.03	43.8±1.1	43.8±1.1	73.0±8.9	61.5±6.4	4.9±0.6	95.1±0.6	n.d.	37.3±0.4	62.0±0.4	0.7±0.1
20	2.1±0.03	54.3±3.8	54.3±4.0	78.1±6.9	71.1±9.6	4.2±0.5	95.8±0.5	n.d.	36.8±0.2	62.3±0.4	0.9±0.1
21	2.0±0.1	13.4±4.3	13.4±4.4	50.7±13.7	27.5±7.7	10.4±1.7	87.6±2.5	1.9±0.8	46.5±1.6	48.1±4.2	5.4±2.6
22	1.6±0.1	3.0±0.4	3.0±0.7	31.3±3.7	10.4±3.1	14.6±2.2	73.7±1.4	11.7±0.9	20.6±3.7	21.9±2.2	57.5±5.5
23	1.7±0.03	9.6±1.9	9.6±3.0	44.4±6.1	22.2±7.5	9.4±1.3	78.8±1.5	11.8±0.3	6.4±0.7	11.9±1.0	81.7±1.8
24	1.7±0.02	19.4±2.7	19.4±4.7	63.8±11.6	32.7±9.0	5.3±0.1	78.6±0.5	16.1±0.5	6.7±0.3	9.7±0.7	83.6±0.9
25	1.7±0.04	24.8±4.2	24.8±7.0	69.9±10.1	37.5±11.2	4.7±0.6	77.9±0.7	17.3±0.7	10.1±0.4	10.8±0.9	79.1±1.3
26	2.0±0.1	29.1±4.8	29.1±7.7	79.8±11.2	37.1±9.6	3.2±0.4	80.7±0.7	16.1±1.1	17.5±2.2	18.8±3.3	63.7±5.5
27	2.7±0.1	17.1±5.7	17.1±6.4	59.7±8.0	29.4±9.8	4.6±0.7	90.2±1.7	5.1±1.2	43.6±0.4	45.6±1.3	10.7±1.2
28	2.9±0.03	27.6±7.4	27.6±7.9	69.6±5.4	39.5±10.2	3.4±0.1	94.3±0.4	2.2±0.4	45.0±0.3	46.7±0.7	8.3±0.9
29	2.9±0.02	40.4±8.8	40.4±9.8	92.9±8.0	42.7±7.7	2.9±0.2	92.5±0.6	4.6±0.4	42.2±0.1	43.9±1.3	13.9±1.3
30	2.7±0.03	29.7±6.5	29.7±8.4	78.9±10.4	38.1±9.5	5.0±0.5	81.1±0.8	14.0±0.5	34.0±1.6	27.1±0.3	38.9±1.8
31	2.6±0.03	31.9±5.7	31.9±7.8	81.8±10.7	39.8±9.3	3.8±0.2	80.2±0.8	16.0±0.9	23.5±1.5	23.4±0.6	53.0±2.0
32	2.5±0.04	40.0±7.8	40.0±11.8	94.6±12.4	42.6±10.9	1.5±0.1	76.8±0.4	21.7±0.4	14.5±0.7	18.8±0.7	66.7±0.4
33	2.8±0.1	32.7±5.7	32.7±8.2	86.9±10.3	38.1±9.4	2.4±0.1	79.2±0.8	18.5±0.9	25.1±0.9	23.3±1.0	51.7±1.9
34	3.4±0.02	30.4±6.1	30.4±7.6	100.2±12.7	30.9±7.4	2.3±0.1	87.1±0.4	10.6±0.4	33.1±0.2	37.1±0.8	29.8±0.8
35	3.5±0.04	44.7±13.0	44.7±15.4	121.8±13.5	34.2±6.3	1.6±0.1	90.9±0.2	7.5±0.01	34.2±0.1	42.2±0.4	23.6±0.4
36	3.5±0.1	44.8±8.4	44.8±10.1	108.2±10.2	40.5±6.0	1.9±0.1	87.3±0.6	10.8±0.6	38.5±0.6	32.3±1.2	29.2±1.4
37	3.5±0.1	43.4±8.0	43.4±10.5	90.3±9.5	47.9±10.7	2.0±0.3	80.2±1.2	17.8±0.9	31.2±1.0	27.2±0.6	41.6±1.5

38	3.5±0.1	52.1±7.5	52.1±10.8	95.8±6.6	53.4±8.8	1.2±0.2	76.0±2.0	22.8±1.9	22.9±0.8	26.1±0.2	51.0±1.0
39	3.8±0.1	55.4±8.4	55.4±11.9	101.9±8.0	53.6±9.6	1.5±0.2	78.8±2.2	19.7±2.0	27.1±0.8	26.2±0.1	46.7±0.8
40	4.1±0.2	56.5±9.9	56.5±12.9	108.5±9.8	52.3±12.0	1.4±0.1	85.3±1.3	13.3±1.3	27.3±1.0	31.5±0.4	41.2±0.6
41	3.9±0.04	69.3±2.9	69.3±4.2	118.2±12.0	59.7±6.2	1.5±0.1	80.9±1.1	17.6±1.1	32.8±0.6	29.9±1.8	37.3±1.2
42	4.0±0.03	61.9±0.8	61.9±0.7	113.8±12.8	55.6±5.5	1.9±0.1	76.1±0.6	22.0±0.7	31.8±0.3	25.8±0.9	42.4±1.2
43	4.0±0.04	69.7±0.8	69.7±1.1	105.1±6.3	66.7±3.0	1.3±0.1	73.8±0.5	24.9±0.5	26.8±0.2	24.2±0.9	49.0±0.8
44	4.3±0.04	68.0±0.9	68.0±1.2	110.2±6.5	62.2±4.0	1.6±0.1	76.1±0.2	22.3±0.3	28.7±0.3	25.9±0.6	45.4±0.2
45	4.5±0.1	80.5±0.8	80.5±0.7	129.6±10.1	62.8±4.4	1.2±0.1	78.1±0.5	20.7±0.5	29.5±0.4	28.8±0.4	41.7±0.7
46	4.6±0.03	77.4±2.0	77.4±2.7	124.2±12.6	63.1±4.1	1.4±0.1	75.1±0.6	23.5±0.6	30.3±0.6	25.8±0.8	43.9±0.6
47	4.7±0.03	83.7±1.0	83.7±1.3	124.0±11.2	68.6±6.1	1.1±0.03	74.0±0.4	24.9±0.3	26.3±0.4	25.7±0.4	48.0±0.2
48	4.9±0.04	82.7±1.7	82.7±2.6	138.6±12.1	60.4±4.9	1.3±0.1	75.2±0.6	23.5±0.6	27.7±1.0	27.1±0.9	45.2±0.5
49	5.1±0.04	87.7±1.8	87.7±2.7	136.6±11.7	65.0±4.8	1.1±0.02	75.3±0.8	23.6±0.8	28.3±0.7	26.9±0.8	44.8±0.4
50	5.2±0.1	89.5±0.2	89.5±0.18	138.6±9.3	65.2±7.5	1.1±0.2	73.3±0.1	25.6±0.2	27.5±0.7	24.5±0.002	48.0±0.7
51	5.4±0.04	85.4±1.6	85.4±2.3	121.3±7.2	70.9±4.6	1.0±0.1	73.9±0.6	25.1±0.5	26.2±0.6	26.8±0.9	46.9±0.7
52	5.8±0.04	81.7±2.3	81.7±3.6	112.1±6.2	73.3±4.9	1.0±0.1	75.1±0.6	23.9±0.6	26.5±0.3	26.9±1.0	46.6±0.6
53	6.0±0.03	72.8±3.7	72.8±5.4	108.9±7.2	66.9±3.4	1.0±0.1	74.7±0.7	24.3±0.7	26.9±0.5	25.6±0.8	47.6±0.4
54	6.2±0.04	75.8±1.2	75.8±2.1	102.4±7.4	74.7±5.7	1.0±0.1	74.4±0.7	24.5±0.6	26.0±0.5	25.7±0.6	48.4±0.4
55	6.6±0.2	69.7±4.1	69.7±6.4	94.1±10.8	74.9±5.5	0.9±0.2	74.8±1.4	24.3±1.3	25.5±0.4	25.8±1.1	48.6±0.8
56	6.5±0.2	60.2±3.7	60.2±4.6	104.1±16.6	62.5±14.6	1.0±0.1	75.9±1.5	23.0±1.4	27.0±1.9	26.3±1.1	46.6±1.5
57	6.8±0.3	56.1±4.4	56.1±5.4	65.8±28.5	59.1±14.1	1.1±0.3	76.1±2.0	22.9±1.7	27.3±2.0	25.1±1.2	47.6±1.9
58	7.0±0.4	57.6±7.3	57.6±9.3	64.5±33.0	61.6±16.9	1.0±0.3	76.7±2.3	22.3±2.0	26.5±2.0	25.5±0.4	48.0±1.6
59	7.3±0.5	51.7±4.3	51.7±5.3	52.9±29.0	58.7±7.9	0.9±0.3	77.1±2.6	22.0±2.3	26.3±2.9	25.4±1.3	48.4±1.9
60	7.6±0.6	49.8±5.4	49.8±6.8	65.0±5.1	79.0±15.6	0.9±0.4	77.3±2.4	21.8±2.1	26.6±2.9	25.2±1.5	48.3±3.0
61	6.8±0.1	41.8±2.0	41.8±3.0	70.4±5.7	60.5±7.8	1.6±0.1	72.5±0.2	25.9±0.2	29.3±0.1	25.6±0.1	45.1±0.1
62	6.8±0.1	39.1±1.4	39.1±2.1	65.0±8.6	62.9±10.4	1.5±0.02	72.5±0.3	25.9±0.3	30.7±0.6	24.3±0.3	45.1±0.7
63	6.8±0.1	38.7±2.3	38.7±3.3	71.8±16.1	60.3±14.2	1.5±0.1	72.2±0.3	26.3±0.3	30.2±0.7	24.7±0.2	45.0±0.7
64	6.9±0.2	35.2±3.3	35.2±4.8	69.2±12.0	55.9±14.5	1.6±0.1	72.5±0.4	26.0±0.3	29.9±1.0	25.7±0.9	44.4±1.1
65	7.0±0.1	34.4±1.3	34.4±2.0	59.7±7.3	59.9±9.3	1.4±0.2	72.8±0.4	25.9±0.4	29.8±1.0	25.6±0.7	44.7±0.4
66	7.0±0.1	33.1±1.4	33.1±2.1	65.0±5.3	51.9±6.6	1.4±0.3	72.8±0.4	25.7±0.4	29.4±1.0	26.0±0.4	44.6±0.6
67	7.4±0.3	33.2±1.7	33.2±2.5	40.3±2.7	83.7±10.6	1.3±0.3	73.7±0.3	25.0±0.1	28.6±1.8	26.5±0.4	44.9±1.7
68	7.4±0.2	31.4±1.5	31.4±2.3	46.8±5.2	69.1±10.1	1.3±0.3	73.4±0.6	25.3±0.6	28.6±1.4	25.5±0.2	45.9±1.1
69	7.2±0.2	30.7±1.6	30.7±2.4	67.7±19.4	52.3±13.6	1.5±0.1	74.4±0.9	24.1±0.8	30.1±1.0	26.3±0.3	43.6±0.8

70	7.5±0.2	78.3±5.2	78.3±7.6	156.7±16.7	51.2±7.5	1.1±0.1	72.7±0.3	26.2±0.4	27.0±0.1	26.0±0.6	47.0±0.7
71	8.0±0.001	482.1±12.0	482.1±17.5	829.8±80.0	58.9±4.3	1.1±0.04	70.8±0.1	28.1±0.1	26.7±0.01	26.2±0.2	47.1±0.2
72	7.5±0.1	934.9±9.5	934.9±14.3	2651.1±191.5	35.7±2.8	1.7±0.02	67.8±0.1	30.5±0.1	23.4±1.4	28.6±0.7	48.0±0.8
73	12.7±0.4	661.5±16.2	661.5±24.5	1265.4±96.6	52.6±2.0	1.0±0.01	68.0±0.2	31.0±0.2	22.1±1.9	28.5±1.0	49.3±1.1
74	15.1±0.6	274.4±5.1	274.4±7.9	527.5±21.7	52.1±0.7	1.0±0.01	69.1±0.3	29.9±0.3	24.0±1.2	29.7±1.2	46.3±2.1
75	14.7±0.6	144.6±4.6	144.6±7.0	250.2±9.6	57.9±3.1	0.7±0.02	70.6±0.6	28.6±-0.6	22.6±1.7	29.8±0.4	47.6±2.1
76	15.0±1.1	90.6±7.1	90.6±9.9	165.2±9.2	55.8±8.7	0.9±0.1	71.1±1.1	28.0±0.9	25.3±1.8	28.9±1.3	45.8±3.1
77	14.8±1.7	67.7±6.8	67.7±9.1	116.2±5.3	57.8±5.9	0.9±0.2	72.9±1.4	26.2±1.3	26.3±1.9	28.7±2.3	45.0±3.5
78	15.4±2.1	52.1±5.1	52.1±6.7	84.4±0.8	61.7±7.7	1.0±0.2	73.9±1.7	25.2±1.5	25.6±2.3	28.5±2.7	45.9±4.3
79	15.8±2.8	46.4±5.0	46.4±6.3	70.4±2.3	66.6±10.7	1.0±0.2	74.6±2.0	24.4±1.8	27.5±1.9	27.2±3.4	45.2±5.1
80	17.3±3.6	40.7±5.1	40.7±6.2	62.6±7.1	68.3±15.4	1.0±0.3	76.4±2.7	22.6±2.5	27.3±2.6	25.2±3.9	47.5±5.9

a = PA concentration in each fraction following HPLC/phloroglucinolysis determined by the summation of individual subunit concentrations determined using published conversion factors (*24*)

b = PA concentration in each fraction following HPLC/phloroglucinolysis determined by the summation of individual subunit concentrations calculated as catechin equivalents

c = PA concentration in each fraction determined by absorbance at 280nm on a UV-Viis spectrophotometer before phloroglucinolysis

abbrev. Cat = Catechin, Ecat = Epicatechin, Ecatgall = Epicatechin gallate, n.d. = not detected.

Table 2. Cabernet Sauvignon seed PA separated by semi-preparative diol phase chromatography to report DP, PA concentration, percent conversion yield and the proportion of extension and terminal subunits in individual fractions. Fraction number corresponds to the time of elution (minutes). PA was not detected in seed fractions prior to Fraction 17.

Cabernet Sauvignon seed											
Fraction #	**DP**	**PA Concentration**			**% Conversion**	**% of Extension subunits**			**% of Terminal subunits**		
		(mg/L HPLC)[a]	(mg/L HPLC CE)[b]	(mg/L UV-Vis CE)[c]	**Yield**	Cat	Ecat	Ecatgall	Cat	Ecat	Ecatgall
17	2.3±0.3	16.2±1.8	17.6±1.8	36.2±4.5	47.6±13.1	1.7±1.0	98.3±1.0	n.d.	28.9±0.5	56.8±0.6	14.4±0.4
18	2.3±0.3	25.1±3.3	26.0±3.4	60.9±0.5	48.1±3.8	2.3±1.4	97.7±1.4	n.d.	35.5±0.7	62.6±0.8	1.9±1.0
19	2.0±0.1	35.6±2.3	36.7±2.4	62.8±11.0	55.2±13.3	3.3±1.7	96.7±1.7	n.d.	41.3±0.8	57.0±0.6	1.7±0.2
20	2.1±0.01	41.4±2.1	43.0±2.2	58.5±9.2	60.8±3.8	3.0±1.5	96.8±1.6	0.3±0.3	39.3±0.7	58.2±0.6	2.4±0.1
21	2.1±0.1	23.1±4.0	24.9±4.4	38.6±7.4	53.2±8.8	6.7±1.3	90.3±1.0	3.1±1.0	48.8±0.5	44.1±0.7	7.2±0.8
22	2.0±0.2	4.7±0.8	6.3±1.3	16.0±8.1	23.9±7.5	8.7±3.0	78.2±1.2	13.1±3.2	25.3±9.2	25.5±1.2	49.2±9.0
23	1.9±0.1	8.4±0.2	12.4±0.4	22.6±8.0	41.7±0.1	7.1±1.0	77.6±1.1	15.3±1.5	14.8±1.5	17.0±1.2	68.2±2.7
24	1.9±0.1	13.3±0.7	20.4±1.0	43.7±11.6	37.5±3.9	4.6±0.5	74.8±0.3	20.6±0.8	13.6±1.2	16.5±0.4	69.9±1.5
25	2.0±0.1	18.3±0.7	27.6±0.8	66.0±10.6	36.7±0.3	2.9±0.7	76.1±1.7	21.1±1.7	15.5±2.5	18.2±2.2	66.3±2.7
26	2.4±0.1	21.6±0.9	29.9±1.8	43.7±5.9	58.2±3.7	2.3±0.7	77.9±1.0	19.8±1.5	26.8±4.3	28.5±2.2	44.7±5.5
27	3.2±0.3	27.5±2.4	31.3±3.0	51.4±11.6	54.2±3.0	2.1±0.5	90.2±1.4	7.7±1.9	46.5±1.1	43.7±1.1	9.9±1.7
28	3.3±0.3	36.1±3.1	40.1±4.2	56.1±8.7	65.6±10.3	2.3±0.3	93.2±1.6	4.5±1.9	48.2±0.3	43.3±0.2	8.5±0.2
29	3.1±0.2	46.0±2.1	52.3±2.6	69.9±14.7	63.0±1.2	2.6±0.1	91.7±0.7	5.8±0.8	47.4±0.6	38.8±0.9	13.7±0.4
30	3.0±0.2	29.3±2.5	36.8±3.3	51.0±7.5	68.7±1.5	4.4±0.4	83.3±0.7	12.3±1.0	41.0±0.1	27.8±0.5	31.2±0.5
31	3.1±0.3	27.2±2.3	37.2±2.7	51.0±9.3	66.4±7.0	3.1±0.6	77.4±1.2	19.5±0.7	31.8±1.2	22.8±1.8	45.4±1.6
32	3.0±0.3	30.2±2.5	44.6±3.5	67.2±10.7	62.5±3.6	1.4±0.2	72.9±1.3	25.6±1.1	20.1±1.2	20.6±2.2	59.2±3.4
33	3.2±0.3	32.5±4.5	44.6±6.7	69.6±15.9	57.8±0.2	2.1±0.4	78.2±1.4	19.7±1.5	29.9±1.8	25.8±1.3	44.2±3.1
34	3.6±0.2	32.3±1.5	38.9±1.6	67.7±12.5	47.8±0.7	2.2±0.2	87.8±0.7	10.0±0.7	38.4±0.9	38.1±0.8	23.6±0.2
35	3.6±0.1	42.9±2.5	50.9±2.9	81.5±7.1	55.9±3.3	1.9±0.2	89.1±0.8	9.0±0.7	39.3±0.9	39.9±1.9	20.8±1.0
36	3.5±0.1	41.9±2.4	51.0±3.1	79.6±5.6	58.7±8.7	2.0±0.3	87.1±0.4	10.9±0.2	44.2±1.0	31.5±1.8	24.2±2.0
37	3.5±0.1	30.2±2.7	39.5±3.7	71.8±12.6	55.9±1.8	1.4±0.4	81.4±0.6	17.2±0.3	36.2±2.3	29.7±0.7	34.1±2.6
38	3.7±0.2	34.3±4.2	47.9±6.1	75.0±8.8	70.2±3.1	1.0±0.2	75.6±0.3	23.5±0.2	29.4±2.0	28.5±0.7	42.1±1.8
39	4.1±0.02	40.8±3.6	54.0±5.0	71.6±5.9	64.1±5.7	1.4±0.2	81.7±2.2	16.9±2.0	29.9±0.4	29.3±1.3	40.8±1.2
40	4.4±0.1	48.3±4.0	60.2±5.1	83.5±12.7	60.9±7.5	1.4±0.2	86.9±1.7	11.8±1.6	32.1±0.3	34.8±1.9	33.1±1.6
41	4.2±0.1	45.7±3.9	58.0±5.0	92.9±4.6	54.8±1.7	1.7±0.2	83.9±0.6	14.4±0.6	39.8±0.8	30.0±1.6	30.1±1.3

42	4.3±0.2	42.7±4.6	56.9±5.5	100.0±11.6	47.3±4.1	1.3±0.3	80.2±1.8	18.5±1.5	32.7±1.5	26.3±0.5	41.0±1.9
43	4.4±0.1	45.9±5.6	64.1±8.0	83.7±6.2	63.8±4.7	1.0±0.2	75.8±0.6	23.2±0.5	29.4±1.1	25.5±0.4	45.0±0.9
44	4.9±0.1	44.5±4.1	58.3±4.2	85.4±10.0	57.5±3.7	1.3±0.1	81.8±2.3	16.9±2.1	31.5±0.5	28.2±1.5	40.2±1.7
45	4.8±0.1	54.3±6.0	71.1±7.7	90.3±13.9	60.9±1.4	1.2±0.2	81.7±0.5	17.2±0.3	35.2±0.9	29.1±0.4	35.7±0.7
46	4.9±0.1	50.2±4.5	67.5±5.5	90.3±7.3	63.6±0.3	1.2±0.3	79.2±1.3	19.6±1.1	32.8±0.8	26.3±0.02	40.9±0.8
47	5.0±0.1	49.8±2.5	68.6±3.6	100.9±8.6	59.7±3.4	1.2±0.2	77.0±0.4	21.7±0.3	30.1±0.3	26.1±0.6	43.8±0.4
48	5.3±0.2	60.2±9.2	80.9±12.4	110.2±18.3	56.8±13.6	1.2±0.3	79.5±0.2	19.3±0.3	29.3±1.7	28.9±0.4	41.7±1.8
49	5.4±0.1	56.2±3.5	76.1±4.8	116.5±11.4	55.8±1.3	1.1±0.1	78.6±0.3	20.3±0.2	32.4±0.3	26.0±0.2	41.6±0.5
50	5.6±0.2	59.8±5.0	82.9±6.9	111.9±10.8	62.0±0.3	1.0±0.1	76.4±0.9	22.6±0.7	29.2±0.2	25.7±0.5	45.1±0.7
51	6.0±0.1	57.8±6.0	78.8±8.3	93.4±9.3	70.8±8.1	0.9±0.1	78.1±0.5	21.0±0.4	29.8±0.1	26.9±1.0	43.3±1.1
52	6.1±0.2	57.7±8.7	78.4±12.1	97.3±14.3	60.0±0.9	0.9±0.1	78.5±0.6	20.6±0.5	30.7±0.1	26.3±0.5	42.9±0.5
53	6.3±0.3	38.1±5.4	52.2±7.0	80.6±9.9	59.1±10.8	1.0±0.2	77.2±0.9	21.8±0.8	30.1±0.8	25.6±0.9	44.3±0.6
54	6.6±0.2	49.1±6.2	67.4±8.5	81.8±11.4	65.7±5.9	0.9±0.2	77.1±1.2	22.0±1.0	29.7±0.3	25.6±0.4	44.6±0.2
55	6.8±0.4	43.8±3.6	59.5±5.0	72.3±11.1	65.5±1.6	1.0±0.2	78.0±1.1	20.9±0.9	30.3±1.1	27.2±0.4	42.5±1.1
56	7.1±0.4	43.5±4.9	58.9±6.6	77.6±10.4	60.9±8.9	1.0±0.2	78.5±1.0	20.5±0.8	30.6±0.8	25.6±0.4	43.8±0.7
57	7.2±0.6	33.3±6.7	54.9±3.9	75.2±8.3	63.9±5.6	1.3±0.3	77.3±1.6	21.4±1.4	31.2±1.5	24.7±1.6	44.1±1.4
58	7.4±0.7	31.4±5.9	51.4±3.2	65.0±12.7	64.2±0.6	1.2±0.3	78.6±1.1	20.2±0.7	30.1±1.5	25.9±0.8	43.9±1.8
59	7.9±1.0	35.6±2.4	47.6±3.1	57.3±6.9	76.6±15.6	1.1±0.4	79.2±1.1	19.7±0.8	30.5±1.1	25.6±1.2	43.9±2.3
60	8.5±1.2	35.2±5.5	47.0±7.3	54.4±4.6	76.2±20.6	1.1±0.4	79.5±1.1	19.4±0.8	30.7±0.9	23.3±2.5	45.9±3.4
61	7.4±0.3	29.6±2.5	39.6±3.6	51.7±7.2	62.8±7.6	1.5±0.2	78.8±1.6	19.7±1.4	32.0±0.8	25.3±1.0	42.7±1.4
62	7.6±0.4	29.8±2.6	39.9±3.1	52.4±8.0	65.4±12.8	1.4±0.2	78.9±1.9	19.7±1.8	32.1±0.6	24.5±2.2	43.4±2.1
63	8.3±0.6	28.8±5.3	38.3±6.2	43.7±8.7	61.3±2.0	1.4±0.4	79.4±2.6	19.2±2.2	26.1±4.9	25.7±0.7	48.2±4.3
64	9.2±1.0	30.4±8.6	39.9±10.2	64.1±18.6	40.5±11.5	1.2±0.4	79.9±3.1	19.0±2.7	26.0±5.5	23.5±2.8	50.5±5.1
65	7.8±0.5	17.6±2.4	23.2±3.4	53.4±12.5	43.8±18.8	1.4±0.2	79.6±2.1	19.0±2.0	33.1±1.1	26.3±0.9	40.6±1.8
66	7.6±0.4	17.3±3.2	22.8±4.2	57.0±19.2	36.6±7.3	1.5±0.2	79.3±1.5	19.2±1.4	33.2±0.8	25.5±1.3	41.3±1.8
67	7.7±0.4	21.3±3.1	28.0±3.9	35.2±6.1	80.6±15.3	1.4±0.2	80.0±1.4	18.6±1.2	32.5±1.3	26.2±0.8	41.3±1.8
68	7.8±0.4	17.5±1.9	23.0±2.3	32.5±6.5	65.0±2.4	1.4±0.2	80.1±1.4	18.5±1.3	32.4±1.9	26.2±1.4	41.4±2.0
69	7.8±0.3	22.3±4.1	29.3±5.7	39.1±12.2	50.5±16.1	1.9±0.5	79.0±1.3	19.1±1.2	31.7±1.3	28.2±0.6	40.1±1.3
70	7.8±0.7	47.6±5.9	64.0±8.4	66.2±12.4	90.0±17.6	1.4±0.2	78.2±0.8	20.4±0.9	30.5±0.9	27.6±0.6	41.9±1.3
71	7.0±0.7	285.4±15.3	397.8±26.5	623.4±15.1	34.5±5.4	1.1±0.2	74.8±1.3	24.1±1.2	34.5±5.5	25.1±2.0	40.4±3.5
72	6.8±0.7	508.8±44.5	715.2±67.4	2273.1±156.3	18.3±1.2	2.2±0.4	72.2±0.5	25.6±0.8	36.6±9.1	26.5±4.3	36.9±4.8
73	10.7±1.3	352.4±23.0	494.1±34.9	1064.5±72.4	24.0±0.7	1.5±0.2	73.3±0.4	25.3±0.6	33.0±7.7	28.4±3.4	38.6±4.5

74	14.3±1.1	174.3±26.4	243.3±36.9	428.3±67.0	17.6±6.7	1.0±0.1	74.4±0.3	24.6±0.3	26.2±0.8	29.4±1.9	44.4±2.2
75	13.7±1.2	81.3±7.3	112.9±10.4	199.2±21.9	50.9±0.9	1.0±0.1	74.7±0.4	24.2±0.2	26.8±1.5	30.8±0.3	42.4±1.8
76	13.6±1.2	55.5±8.8	75.7±11.8	124.0±12.6	52.1±4.3	1.1±0.2	76.2±1.2	22.7±1.0	28.2±1.9	30.1±0.6	41.7±1.8
77	13.2±1.3	49.1±10.1	66.2±13.4	97.3±12.3	54.9±2.7	1.0±0.2	77.5±1.3	21.6±1.1	29.1±1.3	29.7±0.7	41.2±1.0
78	13.6±2.1	43.2±9.0	57.3±11.7	87.6±4.4	54.9±15.9	1.1±0.3	78.5±1.9	20.5±1.7	29.4±2.1	30.6±1.2	40.0±3.2
79	14.6±1.7	37.2±6.5	48.9±8.9	66.5±10.1	59.6±2.5	0.8±0.1	80.6±1.1	18.6±1.1	28.5±0.7	28.2±2.1	43.2±1.6
80	15.1±3.5	43.1±7.1	47.3±9.2	67.9±4.2	56.4±12.5	1.0±0.3	79.7±2.3	19.3±2.1	31.5±1.2	26.0±4.3	42.5±4.5

a = PA concentration in each fraction following HPLC/phloroglucinolysis determined by the summation of individual subunit concentrations determined using published conversion factors (*24*)

b = PA concentration in each fraction following HPLC/phloroglucinolysis determined by the summation of individual subunit concentrations calculated as catechin equivalents

c = PA concentration in each fraction determined by absorbance at 280nm on a UV-Vis spectrophotometer before phloroglucinolysis

abbrev. Cat = Catechin, Ecat = Epicatechin, Ecatgall = Epicatechin gallate, n.d. = not detected.

Table 3. Shiraz skin PA separated by semi-preparative diol phase chromatography to report DP, PA concentration, percent conversion yield and the proportion of extension and terminal subunits in individual fractions. Fraction number corresponds to the time of elution (minutes). PA was not detected in skin fractions prior to Fraction 40.

		Shiraz skin										
Fraction #	**DP**	**PA Concentration**			**% Conversion**	**% of Extension subunits**				**% of Terminal subunits**		
		(mg/L HPLC)[a]	(mg/L HPLC CE)[b]	(mg/L UV-Vis CE)[c]	**Yield**	Epigall	Cat	Ecat	Ecatgall	Cat	Ecat	Ecatgall
40	4.7±0.5	6.3±0.2	5.8±0.1	99.7±7.0	5.9±0.5	22.1±1.7	2.3±0.5	71.3±1.1	4.3±0.4	63.8±4.7	26.2±3.5	9.9±2.4
41	5.4±0.2	6.4±0.4	6.1±0.3	101.2±1.8	6.0±0.2	26.4±3.4	2.0±0.4	63.2±3.5	8.4±1.2	60.4±5.0	26.8±3.8	12.9±1.6
42	5.6±0.1	6.7±1.0	6.7±1.0	102.9±10.0	6.8±1.5	23.6±2.6	1.1±0.6	64.3±3.9	10.9±1.4	59.5±4.9	29.4±6.4	11.1±3.1
43	6.1±0.1	7.3±0.1	7.5±0.1	92.2±1.1	8.1±0.1	21.5±1.2	1.5±0.2	65.6±2.8	11.5±1.4	64.3±5.8	25.4±4.7	10.4±2.9
44	7.5±0.8	8.4±0.4	8.2±0.5	90.5±1.5	9.0±0.4	22.8±1.2	1.4±0.3	68.0±2.4	7.8±1.0	71.7±3.4	18.0±2.1	10.3±1.4
45	6.6±0.6	9.0±0.6	9.0±0.6	84.9±3.7	10.6±0.4	22.0±0.4	1.1±0.6	67.9±1.4	8.9±0.7	71.4±2.8	18.8±4.0	9.8±1.3
46	7.5±0.5	8.4±0.5	8.5±0.5	100.0±15.1	8.7±0.9	23.4±0.7	1.7±0.4	64.3±1.4	10.5±0.3	67.2±2.2	20.8±3.5	12.0±2.7
47	7.7±0.2	9.8±0.6	9.7±0.6	77.6±1.7	12.5±0.7	26.2±0.5	1.4±0.7	62.0±0.6	10.4±0.5	68.4±3.2	18.8±2.5	12.8±1.3
48	7.0±0.6	9.9±0.3	10.0±0.4	87.4±13.1	11.8±1.4	24.2±1.8	1.4±0.7	63.8±0.9	10.5±0.4	71.9±5.3	17.6±3.7	10.5±1.9
49	7.4±1.0	12.3±0.6	12.2±0.7	76.9±3.1	16.0±1.4	23.7±0.7	1.3±0.7	65.7±0.7	9.2±0.6	71.4±3.3	19.2±1.6	9.4±1.6
50	7.8±0.9	11.6±0.4	11.6±0.5	73.8±2.8	15.8±0.4	23.6±0.2	2.3±0.3	64.3±0.6	9.7±0.7	70.4±3.4	19.5±1.5	10.0±1.9
51	8.8±1.2	11.9±0.2	11.8±0.3	69.6±7.8	17.3±1.6	24.4±0.4	2.3±0.1	63.5±1.0	9.7±0.8	68.8±5.5	20.1±2.6	11.2±3.1
52	8.6±0.7	10.8±0.4	10.7±0.5	58.7±3.0	18.3±0.7	23.9±0.5	2.5±0.2	63.9±1.2	9.8±0.9	67.0±4.1	21.2±1.6	11.8±2.6
53	9.0±1.1	11.3±1.0	11.1±1.1	51.2±2.9	21.6±2.0	25.6±0.4	2.6±0.4	62.4±0.2	9.4±0.5	71.5±4.4	19.0±3.0	9.5±1.4
54	8.7±0.8	10.0±0.3	9.8±0.3	48.8±2.1	20.2±1.3	25.0±0.7	2.7±0.2	62.6±1.1	9.7±0.5	71.9±4.4	18.9±3.0	9.3±1.4
55	9.0±0.8	10.4±0.5	10.3±0.6	45.6±3.6	22.6±1.3	25.5±0.2	2.7±0.2	62.0±0.7	9.8±0.6	70.9±4.1	19.3±2.7	9.8±1.4
56	9.6±0.9	10.5±0.4	10.3±0.5	42.9±4.0	24.1±1.7	26.1±0.5	2.9±0.3	61.0±0.1	9.9±0.6	71.0±4.1	18.8±2.5	10.2±1.6
57	10.7±0.7	9.4±0.8	9.3±0.8	38.1±0.9	24.4±2.7	26.1±0.1	3.3±0.4	60.3±0.2	10.3±0.3	68.6±1.7	20.4±1.2	11.0±0.6
58	10.7±0.8	9.5±0.3	9.3±0.4	34.0±1.3	27.4±0.2	26.1±0.3	3.0±0.2	60.9±0.5	10.0±0.4	69.9±1.0	19.5±1.0	10.7±0.2
59	10.8±0.7	8.7±0.5	8.5±0.4	34.5±2.4	25.0±2.7	26.1±0.6	3.0±0.3	60.8±0.9	10.0±0.3	68.2±2.6	21.0±2.5	10.8±0.1
60	12.5±0.3	8.6±0.6	8.4±0.6	33.2±0.9	25.3±1.9	25.9±0.5	3.2±0.4	61.2±0.7	9.8±0.4	64.4±3.1	22.8±2.3	12.8±0.8
61	13.1±0.8	9.0±0.1	8.7±0.1	26.4±1.4	33.0±1.7	26.8±0.5	3.0±0.4	60.4±1.1	9.8±0.3	64.0±2.0	23.6±1.7	12.4±0.4
62	13.5±0.9	7.9±0.6	7.7±0.6	24.3±1.3	31.6±1.8	26.4±0.9	3.0±0.4	60.9±1.4	9.8±0.4	62.3±2.8	24.6±2.0	13.1±0.9
63	13.1±1.7	8.1±0.3	7.9±0.3	24.0±2.6	33.7±4.5	25.9±1.1	3.0±0.4	61.4±1.5	9.7±0.3	64.1±4.8	24.0±3.4	11.9±1.5
64	14.8±2.8	8.8±0.9	8.7±1.0	24.5±2.4	35.4±1.1	23.1±2.8	2.9±0.6	65.0±4.1	9.0±0.7	61.1±6.7	26.8±5.3	12.1±1.6

65	11.6±1.1	7.7±0.2	7.4±0.3	23.8±0.5	31.2±0.9	24.8±1.6	3.1±0.3	62.8±2.3	9.2±0.6	65.5±5.5	23.9±3.8	10.6±1.8
66	12.5±1.5	7.5±0.1	7.3±0.1	22.6±2.2	33.0±3.5	25.0±1.3	3.1±0.3	62.5±2.2	9.4±0.7	63.3±5.6	25.3±3.4	11.4±2.2
67	13.6±1.5	7.6±0.1	7.3±0.1	20.1±1.0	36.4±1.6	24.5±1.7	3.0±0.2	63.4±3.0	9.1±1.0	60.5±6.0	27.5±3.8	12.0±2.3
68	12.5±1.0	7.2±0.2	6.9±0.2	22.6±1.3	30.5±1.0	26.0±1.5	3.2±0.2	61.4±2.6	9.3±0.8	63.0±4.0	25.6±2.3	11.3±1.9
69	15.4±2.5	7.4±0.6	7.1±0.6	16.0±1.3	45.4±7.2	25.6±2.7	2.9±	62.4±4.0	9.0±1.0	61.5±2.4	25.8±2.2	12.7±0.3
70	17.1±3.8	15.9±0.8	15.7±0.7	41.7±2.7	38.0±4.0	25.9±1.2	2.9±	61.4±2.0	9.8±0.7	74.9±6.2	14.5±7.2	10.7±1.0
71	15.1±0.7	85.1±4.0	85.4±4.5	240.5±2.8	35.6±2.2	29.8±1.6	3.6±	54.8±1.3	11.8±0.1	71.6±4.0	20.5±4.0	7.9±0.2
72	14.1±1.0	145.4±3.0	138.1±2.1	697.1±9.1	19.8±0.5	43.5±1.6	3.9±	37.8±2.1	14.8±0.3	69.7±0.2	22.7±0.1	7.6±0.2
73	31.3±0.7	247.8±6.1	227.7±4.1	631.1±26.9	36.3±2.3	40.1±0.7	5.4±	43.4±1.0	11.1±0.1	68.4±2.2	25.0±1.3	6.6±1.8
74	52.8±2.0	209.2±12.0	184.1±9.1	521.0±50.0	36.2±4.4	40.9±1.0	5.6±	44.5±1.3	9.0±0.1	67.2±2.6	31.0±2.1	1.8±0.9
75	56.3±7.0	174.4±8.0	151.0±5.5	344.8±10.1	43.9±2.2	41.1±0.9	5.5±	45.3±1.1	8.1±0.1	62.2±4.8	36.2±4.4	1.6±0.8
76	62.1±7.3	128.6±2.9	109.9±2.5	265.7±7.7	41.5±2.1	41.8±0.9	5.0±	45.6±0.5	7.7±0.1	63.4±0.6	36.6±0.6	n.d.
77	55.8±4.1	114.9±5.4	96.1±3.5	191.4±4.4	50.2±2.0	43.3±1.3	4.8±	44.7±1.4	7.3±0.1	64.8±1.7	35.2±1.7	n.d.
78	54.2±3.3	97.7±4.8	82.5±3.5	160.6±8.9	51.7±4.0	42.2±0.9	4.3±	46.2±1.1	7.3±0.04	63.3±2.3	36.7±2.3	n.d.
79	59.2±3.9	75.8±0.4	63.6±1.0	121.3±5.5	52.7±3.1	42.5±1.1	4.1±	46.2±1.2	7.2±0.2	61.3±2.0	38.7±2.0	n.d.
80	63.1±4.2	66.4±4.5	54.6±2.4	106.3±2.9	51.5±3.7	43.5±1.7	4.2±	45.6±1.3	6.7±0.6	61.0±2.2	39.0±2.2	n.d.

a = PA concentration in each fraction following HPLC/phloroglucinolysis determined by the summation of individual subunit concentrations determined using published conversion factors (*24*)

b = PA concentration in each fraction following HPLC/phloroglucinolysis determined by the summation of individual subunit concentrations calculated as catechin equivalents

c = PA concentration in each fraction determined by absorbance at 280nm on a UV-Vis spectrophotometer before phloroglucinolysis

abbrev. Epigall = Epigallocatechin, Cat = Catechin, Ecat = Epicatechin, Ecatgall = Epicatechin gallate, n.d. = not detected.

Table 4. Cabernet Sauvignon skin PA separated by semi-preparative diol phase chromatography to report DP, PA concentration, percent conversion yield and the proportion of extension and terminal subunits in individual fractions. Fraction number corresponds to the time of elution (minutes). PA was not detected in skin fractions prior to Fraction 40.

Cabernet Sauvignon skin												
Fraction #	**DP**	**PA Concentration**			**% Conversion**	**% of Extension subunits**				**% of Terminal subunits**		
		(mg/L HPLC)[a]	(mg/L HPLC CE)[b]	(mg/L UV-Vis CE)[c]	**Yield**	Epigall	Cat	Epicat	Epicatgall	Cat	Ecat	Epicatgall
40	6.5±1.3	7.2±0.6	6.0±0.6	66.7±10.9	7.5±1.7	30.4±1.5	2.0±0.1	66.2±1.6	1.4±0.1	71.1±1.3	22.9±0.8	6.0±1.5
41	6.4±0.9	7.7±1.0	6.5±0.9	73.5±6.9	7.1±0.2	30.7±1.4	2.6±0.3	64.3±1.7	2.4±1.3	70.5±2.2	22.1±1.9	7.4±0.4
42	5.0±0.9	7.1±0.4	6.2±0.4	68.2±9.3	8.6±0.2	32.2±0.2	3.3±0.9	59.8±0.8	4.7±1.1	74.0±3.1	20.2±1.8	5.8±1.3
43	4.8±0.7	7.1±0.5	6.3±0.5	63.6±7.8	9.7±0.5	28.8±1.4	3.4±0.9	62.8±1.8	5.1±1.1	75.0±2.1	19.0±0.9	6.0±1.2
44	5.2±0.6	8.2±0.2	7.3±0.2	65.0±9.3	10.3±0.8	31.1±0.6	3.1±0.6	61.1±0.5	4.8±0.6	76.2±1.6	18.0±0.6	5.8±1.1
45	6.9±0.9	10.4±1.8	9.4±1.7	65.3±10.3	10.6±2.0	28.0±2.0	2.5±0.2	65.0±2.8	4.5±0.6	77.3±3.1	15.6±2.2	7.1±1.1
46	7.2±0.9	11.0±1.5	9.8±1.4	66.0±8.6	11.4±0.4	29.9±1.8	3.0±0.4	62.6±2.6	4.5±0.6	78.2±2.5	16.1±2.6	5.7±0.2
47	8.0±1.2	12.0±1.6	10.7±1.5	62.1±9.7	13.4±2.0	30.8±2.3	2.3±0.8	61.9±2.9	5.0±0.7	77.5±3.4	18.1±4.2	4.4±0.9
48	7.9±1.1	13.3±2.3	12.0±2.0	73.3±14.0	11.7±1.6	28.1±0.5	2.3±0.1	65.2±1.2	4.4±0.6	77.0±2.5	17.5±2.2	5.5±0.3
49	7.9±0.2	12.6±0.8	11.3±0.6	62.6±9.2	15.6±1.7	29.7±0.2	2.7±0.1	63.1±0.6	4.6±0.5	78.4±3.2	16.4±2.7	5.2±0.5
50	8.3±0.3	13.8±0.8	12.3±0.7	65.0±5.0	17.7±1.0	30.4±1.0	2.8±0.4	62.3±1.0	4.5±0.3	78.0±3.7	17.0±3.2	5.0±0.5
51	9.5±1.0	13.6±1.5	12.1±1.3	54.8±10.3	19.3±0.8	29.4±0.9	2.4±0.4	63.7±1.7	4.5±0.5	77.7±3.9	17.9±4.0	4.4±0.8
52	8.3±0.3	11.4±0.8	10.0±0.7	46.3±10.3	17.4±1.5	32.1±0.5	3.1±0.1	60.0±0.8	4.8±0.3	79.3±2.6	15.7±2.3	5.0±0.4
53	8.6±0.3	11.6±0.6	10.1±0.5	42.9±9.9	19.1±2.1	33.4±1.3	2.7±0.2	58.9±1.3	4.9±0.1	79.2±4.0	15.7±3.5	5.1±0.5
54	11.0±1.8	12.4±1.4	10.8±1.3	41.2±8.6	19.4±0.3	31.9±2.1	2.8±0.2	60.9±2.7	4.4±0.4	77.8±3.2	16.3±3.1	5.9±0.3
55	9.7±0.7	10.9±0.8	9.3±0.7	42.9±7.0	18.2±1.6	34.2±0.8	2.9±0.2	58.2±1.2	4.7±0.2	75.6±0.9	18.6±0.6	5.8±0.3
56	12.4±2.6	11.0±1.4	9.5±1.3	43.2±14.3	16.9±4.2	32.7±2.4	2.9±0.3	60.1±3.3	4.3±0.6	77.6±4.9	18.1±2.7	4.4±2.2
57	14.1±2.3	10.3±0.7	8.8±0.7	37.1±12.4	18.4±4.9	33.8±1.3	2.7±0.1	59.4±1.4	4.1±0.3	73.7±3.8	18.0±4.6	8.3±1.2
58	11.1±0.8	10.5±0.6	9.0±0.5	34.7±10.0	21.1±4.6	34.3±0.9	3.2±0.4	57.8±1.3	4.7±0.2	78.4±5.9	17.0±3.6	4.6±2.3
59	10.9±1.2	9.7±0.9	8.3±0.8	31.5±11.8	19.8±3.9	33.8±1.2	3.2±0.5	58.5±1.8	4.6±0.2	73.0±2.3	20.1±2.1	6.9±0.3
60	10.6±0.8	9.1±0.7	7.7±0.7	31.3±11.7	19.1±4.6	34.3±1.5	3.1±0.4	58.0±1.9	4.6±0.2	72.1±1.5	20.9±1.3	7.0±0.3
61	13.1±1.5	9.1±0.6	7.8±0.6	29.1±8.6	20.2±3.1	32.1±2.2	2.8±0.3	61.5±3.2	3.6±0.7	71.5±1.7	21.2±1.3	7.2±0.4
62	11.1±0.8	8.4±0.7	7.0±0.7	31.1±10.6	20.2±3.8	35.5±1.0	2.9±0.3	57.3±0.5	4.4±0.4	71.7±3.2	22.8±0.6	5.5±2.7
63	10.7±0.6	7.7±0.8	6.4±0.7	28.9±10.9	19.1±3.2	35.8±0.7	3.0±0.2	56.9±0.7	4.3±0.4	69.9±0.9	22.2±0.7	8.0±0.3
64	10.8±0.8	6.9±0.1	5.7±0.01	30.6±8.7	15.2±2.3	35.0±0.5	3.0±0.2	57.7±0.5	4.3±0.4	68.3±3.0	23.5±2.0	8.2±0.9

65	12.3±0.2	5.2±2.6	4.4±2.2	32.5±12.3	10.7±3.1	35.4±0.5	2.8±0.4	57.3±0.1	4.5±0.2	71.0±4.8	24.6±0.4	4.4±4.4
66	12.6±0.4	7.9±0.4	6.6±0.4	28.9±12.4	17.3±4.0	35.5±0.2	3.1±0.2	57.0±0.4	4.5±0.1	65.4±0.3	25.9±0.4	8.7±0.1
67	11.2±1.2	6.3±0.8	5.2±0.7	28.9±10.2	18.7±6.3	36.1±0.6	2.9±0.4	56.8±0.8	4.2±0.3	67.9±2.1	27.0±0.5	5.1±2.6
68	11.4±0.6	6.6±0.3	5.4±0.3	27.4±11.5	17.3±5.1	36.3±1.2	2.8±0.4	56.9±1.0	4.0±0.3	67.6±3.5	27.1±0.8	5.4±2.7
69	13.7±2.5	7.2±0.5	5.9±0.4	24.3±10.8	19.1±6.5	36.9±0.8	3.0±0.3	55.7±1.1	4.4±0.1	66.7±1.3	28.0±3.5	5.3±2.6
70	14.5±0.8	17.0±0.9	14.2±0.8	47.6±10.0	24.2±4.1	37.6±0.8	3.1±0.3	55.0±0.5	4.3±0.1	75.6±2.3	20.6±1.5	3.8±1.9
71	14.9±0.3	82.7±1.5	71.4±1.1	248.2±20.9	27.8±2.5	37.2±0.3	4.5±0.1	53.2±0.2	5.0±0.1	78.9±0.8	17.2±0.6	4.0±0.2
72	15.5±0.6	154.9±3.1	118.3±1.6	625.1±11.6	18.5±0.7	55.8±1.0	4.0±0.2	33.0±1.0	7.3±0.1	79.7±0.6	16.2±0.6	4.1±0.3
73	29.4±4.9	263.0±7.7	199.0±4.8	574.8±60.1	30.9±2.0	50.2±0.6	5.6±0.4	39.6±0.5	4.5±0.01	82.3±3.2	16.5±3.6	1.2±0.6
74	42.1±1.8	243.3±10.3	177.9±6.4	485.5±7.1	36.1±2.9	51.8±0.9	5.1±0.2	39.3±1.0	3.7±0.1	77.6±2.3	20.5±2.4	1.9±1.2
75	53.2±6.0	191.3±18.7	139.0±14.7	379.5±21.0	31.9±4.5	51.6±1.4	4.1±0.7	40.9±0.8	3.3±0.03	71.1±4.3	28.9±4.3	n.d.
76	48.4±4.6	162.9±10.9	119.1±7.7	288.5±13.8	37.1±0.7	50.4±0.4	4.7±0.1	41.9±0.4	3.1±0.02	68.8±1.0	31.2±1.0	n.d.
77	76.0±4.9	130.9±7.4	94.6±5.6	213.0±9.7	40.0±0.7	51.0±1.4	3.8±0.6	42.2±0.8	3.0±0.02	65.3±10.3	34.7±10.3	n.d.
78	66.4±4.2	110.8±3.7	79.9±3.0	167.2±8.7	44.0±0.9	51.0±1.2	3.6±0.5	42.5±0.6	2.9±0.01	58.8±7.6	41.2±7.6	n.d.
79	64.9±4.3	80.5±12.1	57.0±8.3	135.4±6.7	46.0±0.4	52.3±1.0	2.8±0.6	42.0±0.7	2.9±0.1	57.4±6.9	42.6±6.9	n.d.
80	55.1±2.9	76.1±2.5	54.2±1.8	135.8±5.5	40.1±2.9	51.5±0.2	3.0±0.4	42.9±0.7	2.6±0.1	60.1±1.9	39.9±1.9	n.d.

a = PA concentration in each fraction following HPLC/phloroglucinolysis determined by the summation of individual subunit concentrations determined using published conversion factors (*24*)

b = PA concentration in each fraction following HPLC/phloroglucinolysis determined by the summation of individual subunit concentrations calculated as catechin equivalents

c = PA concentration in each fraction determined by absorbance at 280nm on a UV-Vis spectrophotometer before phloroglucinolysis

abbrev. Epigall = Epigallocatechin, Cat = Catechin, Ecat = Epicatechin, Ecatgall = Epicatechin gallate, n.d. = not detected.

Table 5. Shiraz wine PA separated by semi-preparative diol phase chromatography to report DP, PA concentration, percent conversion yield and the proportion of extension and terminal subunits in individual fractions. Fraction number corresponds to the time of elution (minutes). PA was not detected in wine fractions prior to Fraction 41.

Shiraz wine											
Fraction #	**DP**	**PA Concentration**			**% Conversion**	**% of Extension subunits**				**% of Terminal subunits**	
		(mg/L HPLC)[a]	(mg/L HPLC CE)[b]	(mg/L UV-Vis CE)[c]	**Yield**	Epigall	Cat	Ecat	Ecatgall	Cat	Ecat
41	6.9±0.3	13.3±0.9	11.3±0.8	244.6±42.0	4.8±0.5	34.7±2.1	3.0±0.2	59.2±2.0	3.1±0.3	77.6±2.9	22.4±2.9
42	7.1±0.5	12.3±1.2	10.9±1.0	277.1±41.1	4.1±0.8	30.0±2.1	2.7±0.4	63.3±2.0	4.0±0.3	83.8±0.5	16.2±0.5
43	7.5±0.4	12.3±1.1	11.2±0.9	230.5±12.8	4.9±0.6	27.3±1.4	2.6±0.4	65.1±0.8	5.0±0.6	83.6±0.5	16.4±0.5
44	8.1±0.6	12.9±1.2	11.3±0.9	222.3±36.0	5.3±0.7	30.3±2.3	2.6±0.4	63.4±2.1	3.7±0.2	82.9±0.8	17.1±0.8
45	8.1±0.4	13.0±0.5	11.4±0.6	173.2±18.1	6.7±0.4	30.2±1.0	2.1±0.3	63.9±1.0	3.8±0.4	82.9±0.4	17.1±0.4
46	8.7±0.6	13.8±0.7	12.1±0.6	176.2±15.0	6.9±0.3	30.8±2.3	2.4±0.3	62.5±1.3	4.3±0.8	83.0±0.7	17.0±0.7
47	10.3±0.8	15.8±1.4	14.0±1.2	152.9±23.5	9.3±0.7	30.4±0.1	2.5±0.3	63.2±0.8	3.9±0.5	83.2±0.4	16.8±0.4
48	11.2±0.7	16.0±1.1	14.2±0.9	244.1±69.3	6.6±1.5	29.4±0.3	1.9±0.3	64.7±0.6	3.9±0.6	82.1±0.4	17.9±0.4
49	10.8±0.6	14.3±1.0	12.6±0.8	164.3±16.7	7.7±0.5	31.1±0.5	2.2±0.3	62.6±0.6	4.2±0.5	81.6±0.3	18.4±0.3
50	11.3±0.6	14.3±0.9	12.6±0.8	155.3±19.3	8.3±0.6	30.0±0.7	2.1±0.4	63.7±0.2	4.1±0.5	80.7±0.2	19.3±0.2
51	11.6±0.5	13.6±0.8	11.6±0.7	125.9±14.9	9.4±0.6	33.1±0.4	2.8±0.3	60.1±0.3	4.0±0.1	82.3±0.9	17.7±0.9
52	11.1±0.7	11.3±0.5	9.9±0.3	134.2±22.5	7.8±1.3	30.9±2.4	2.9±0.4	61.7±1.8	4.5±0.3	81.2±0.6	18.8±0.6
53	11.2±0.3	11.1±0.1	9.5±0.1	155.8±58.5	8.0±2.7	33.7±0.5	2.7±0.3	59.3±0.2	4.3±0.2	79.5±0.5	20.5±0.5
54	11.2±0.2	9.6±0.2	8.2±0.01	90.0±12.1	9.5±1.2	32.0±2.3	2.4±0.4	61.2±2.4	4.3±0.2	77.3±1.1	22.7±1.1
55	11.2±0.5	9.6±0.5	8.3±0.2	82.3±18.2	10.3±2.1	31.1±2.9	2.2±0.3	62.4±2.3	4.3±0.3	76.5±1.9	23.5±1.9
56	11.2±0.6	9.0±0.6	7.7±0.4	100.5±17.2	8.1±1.2	32.5±2.7	2.4±0.4	60.7±2.1	4.4±0.3	75.3±1.9	24.7±1.9
57	10.8±0.2	8.0±0.4	7.0±0.2	89.1±12.8	8.2±1.2	29.4±2.7	2.2±0.3	63.8±2.3	4.6±0.3	73.7±2.5	26.3±2.5
58	11.0±0.2	7.9±0.3	6.8±0.1	72.4±17.8	9.8±2.3	30.9±2.7	2.2±0.1	62.3±2.5	4.6±0.3	73.0±3.2	27.0±3.2
59	11.1±0.2	7.1±0.1	6.2±0.2	64.4±14.9	10.4±2.8	28.6±2.6	2.3±0.1	64.6±2.3	4.5±0.3	72.6±2.6	27.4±2.6
60	11.2±0.2	6.6±0.04	5.8±0.2	73.8±14.4	8.5±1.6	28.2±2.5	2.1±0.1	65.3±2.2	4.4±0.2	71.6±2.7	28.4±2.7
61	9.5±0.2	5.3±0.9	4.4±0.8	68.2±16.1	7.1±2.1	36.0±1.1	3.3±0.3	56.2±1.1	4.5±0.1	70.5±3.8	29.5±3.8
62	9.5±0.3	4.8±0.7	4.0±0.7	57.5±11.6	7.6±2.2	32.9±3.1	3.2±0.4	59.2±2.7	4.7±0.3	69.4±3.3	30.6±3.3
63	9.6±0.2	4.4±0.6	3.6±0.6	47.8±9.7	8.6±2.7	33.1±2.6	3.3±0.5	59.0±2.5	4.7±0.2	68.9±3.3	31.1±3.3
64	9.4±0.2	4.2±0.7	3.5±0.7	48.8±10.2	6.6±2.8	34.0±1.9	3.3±0.4	58.3±2.0	4.5±0.1	69.6±3.7	30.4±3.7
65	9.6±0.2	3.9±0.6	3.2±0.6	47.7±11.3	6.5±3.0	31.8±3.1	3.3±0.4	60.4±3.0	4.5±0.2	68.2±3.0	31.8±3.0

66	9.9±0.3	3.9±0.6	3.3±0.6	42.9±5.8	7.7±3.5	31.3±3.2	3.8±0.1	60.2±2.8	4.8±0.3	69.0±3.5	31.0±3.5
67	9.9±0.3	3.7±0.6	3.1±0.6	43.9±9.6	7.9±2.7	30.8±3.3	3.7±0.1	60.7±2.9	4.8±0.4	68.3±3.4	31.7±3.4
68	10.0±0.3	3.6±0.6	3.0±0.6	54.2±9.8	5.2±0.9	30.5±3.2	3.7±0.2	61.0±2.9	4.8±0.3	67.9±3.2	32.1±3.2
69	10.0±0.5	3.4±0.6	2.8±0.6	32.0±12.4	9.5±5.5	30.3±2.9	3.5±0.3	61.4±2.8	4.8±0.3	67.3±3.1	32.7±3.1
70	14.0±0.5	8.4±1.4	7.5±1.5	71.7±17.8	10.0±4.7	28.7±3.3	2.7±0.2	63.5±2.8	5.1±0.4	72.9±2.9	27.1±2.9
71	18.3±1.2	42. 5±3.4	36.5±2.9	265.3±33.8	13.1±0.2	35.7±0.3	1.6±0.4	57.5±0.5	5.1±0.2	74.7±1.9	25.3±1.9
72	18.2±0.4	45.0±2.9	39.0±2.7	351.6±15.7	11.1±0.3	35.2±0.4	1.6±0.4	57.8±0.1	5.3±0.1	76.3±2.4	23.7±2.4
73	17.2±1.6	22.2±1.8	18.7±1.6	205.0±15.2	9.1±0.4	34.0±0.4	1.8±0.2	60.4±0.4	3.8±0.1	70.9±3.4	29.1±3.4
74	15.5±0.7	12.2±1.2	10.0±1.0	156.7±22.0	6.5±0.4	33.2±0.5	2.1±0.1	61.0±0.7	3.6±0.2	65.5±2.8	34.5±2.8
75	12.2±1.2	6.9±1.3	5.9±1.1	107.7±32.4	7.2±3.1	31.5±1.2	2.2±0.2	62.2±1.4	4.0±0.1	71.2±3.0	28.8±3.0
76	10.5±0.6	5.9±1.3	5.2±1.2	108.2±17.6	5.0±1.3	29.4±2.2	3.3±0.1	62.4±2.1	4.9±0.1	75.7±1.0	24.3±1.0
77	11.2±0.2	5.3±0.8	4.6±0.8	79.7±15.7	5.1±1.5	29.8±2.9	3.4±0.1	61.8±2.9	5.0±0.04	74.4±1.4	25.6±1.4
78	10.3±0.1	3.7±0.5	3.1±0.5	48.5±6.3	6.8±1.8	29.4±3.0	3.6±0.2	62.1±3.0	4.9±0.1	70.0±2.6	30.0±2.6
79	9.6±0.6	2.9±0.5	2.4±0.5	42.5±3.8	6.0±1.6	27.1±3.1	3.6±0.4	64.3±3.3	5.1±0.2	67.7±2.4	32.3±2.4
80	8.6±0.4	2.7±0.4	1.9±0.4	98.3±67.3	4.3±2.2	26.7±3.0	3.9±0.4	64.4±3.4	5.0±0.2	65.7±3.8	34.3±3.8

a = PA concentration in each fraction following HPLC/phloroglucinolysis determined by the summation of individual subunit concentrations determined using published conversion factors (*24*)

b = PA concentration in each fraction following HPLC/phloroglucinolysis determined by the summation of individual subunit concentrations calculated as catechin equivalents

c = PA concentration in each fraction determined by absorbance at 280nm on a UV-Vis spectrophotometer before phloroglucinolysis

abbrev. Epigall = Epigallocatechin, Cat = Catechin, Ecat = Epicatechin, Ecatgall = Epicatechin gallate, n.d. = not detected.

Table 6. Cabernet Sauvignon wine PA separated by semi-preparative diol phase chromatography to report DP, PA concentration, percent conversion yield and the proportion of extension and terminal subunits in individual fractions. Fraction number corresponds to the time of elution (minutes). PA was not detected in wine fractions prior to Fraction 41.

Cabernet Sauvignon wine											
Fraction #	**DP**	**PA Concentration**			**% Conversion**	**% of Extension subunits**				**% of Terminal subunits**	
		(mg/L HPLC)[a]	(mg/L HPLC CE)[b]	(mg/L UV-Vis CE)[c]	**Yield**	Epigall	Cat	Ecat	Ecatgall	Cat	Ecat
41	5.4±0.1	19.2±1.7	16.7±1.4	194.8±12.6	8.6±0.2	32.0±2.3	4.3±0.3	61.1±1.8	2.6±0.5	81.6±1.9	18.4±1.9
42	5.8±0.2	18.4±1.4	15.9±1.0	157.7±36.3	11.3±2.7	32.2±2.3	4.0±0.3	61.1±1.7	2.7±0.3	81.8±0.8	18.2±0.8
43	6.3±0.2	19.2±1.9	16.5±1.4	171.5±14.5	9.6±0.1	32.9±2.1	4.1±0.3	60.4±1.9	2.6±0.1	81.9±0.5	18.1±0.5
44	6.4±0.2	21.0±2.1	18.0±1.6	158.0±29.1	11.9±1.5	33.1±1.9	4.0±0.2	60.4±1.6	2.5±0.2	81.3±1.1	18.7±1.1
45	6.8±0.3	19.9±2.7	17.1±2.1	167.9±16.2	10.1±0.3	32.5±2.7	4.0±0.5	61.1±2.1	2.5±0.2	81.3±1.6	18.7±1.6
46	7.4±0.4	20.8±2.4	17.8±1.8	162.8±16.6	10.9±0.1	32.7±3.1	4.3±0.4	60.4±2.9	2.7±0.05	82.8±1.6	17.2±1.6
47	7.8±0.3	20.7±2.2	17.9±1.7	182.5±10.8	9.8±1.0	32.1±2.1	3.7±0.2	61.4±1.9	2.8±0.1	83.2±1.9	16.8±1.9
48	8.0±0.4	19.2±1.5	16.7±0.9	174.2±11.5	9.6±0.2	30.7±2.6	3.6±0.3	62.8±2.0	2.8±0.2	81.8±2.2	18.2±2.2
49	9.0±0.5	21.1±1.1	18.0±0.7	158.4±15.1	11.5±0.9	33.5±1.2	3.1±0.4	60.8±0.7	2.7±0.2	81.7±1.7	18.3±1.7
50	9.3±0.3	21.5±0.3	18.3±0.3	141.2±7.9	13.0±0.7	33.4±1.1	2.9±0.1	60.9±1.1	2.8±0.1	82.3±2.0	17.7±2.0
51	8.9±0.2	17.9±2.1	14.7±1.8	127.1±11.1	11.5±0.4	37.1±0.3	2.9±0.03	57.4±0.2	2.6±0.1	79.9±2.1	20.1±2.1
52	9.5±0.2	16.0±0.4	13.1±0.3	109.9±6.2	12.0±0.4	37.7±0.05	3.2±0.4	56.5±0.4	2.6±0.1	80.7±1.9	19.3±1.9
53	9.4±0.4	13.5±1.1	11.4±1.1	98.3±6.5	11.5±0.5	34.5±1.8	2.9±0.1	59.7±1.7	3.0±0.1	80.8±2.0	19.2±2.0
54	9.5±0.4	13.3±1.2	11.0±1.0	98.0±3.2	11.1±0.8	36.4±2.0	2.7±0.4	58.2±2.1	2.7±0.2	79.4±2.1	20.6±2.1
55	9.6±0.3	12.4±1.0	10.2±0.9	89.3±4.3	11.3±0.5	36.5±2.4	2.8±0.4	58.1±2.4	2.7±0.2	78.4±1.8	21.6±1.8
56	9.6±0.3	11.5±1.0	9.5±0.6	83.0±5.1	11.4±0.3	35.5±2.5	2.8±0.5	58.6±2.4	3.0±0.4	78.0±2.0	22.0±2.0
57	9.5±0.3	10.3±0.7	8.6±0.4	76.4±5.3	11.2±0.3	35.2±2.3	2.9±0.4	58.8±2.1	3.1±0.5	77.2±2.4	22.8±2.4
58	9.5±0.2	9.4±0.6	7.8±0.3	68.7±3.8	11.4±0.3	34.3±2.6	3.0±0.6	59.5±2.3	3.2±0.6	76.2±2.1	23.8±2.1
59	9.7±0.6	9.1±0.7	7.5±0.5	56.5±4.8	13.5±1.6	34.6±2.5	2.5±0.2	59.6±2.2	3.2±0.6	75.8±2.1	24.2±2.1
60	9.8±0.8	8.7±0.7	7.2±0.5	59.4±2.3	12.2±0.8	34.0±2.5	2.7±0.4	60.3±2.2	3.0±0.3	74.2±2.7	25.8±2.7
61	10.1±0.1	6.8±1.0	5.5±1.0	59.0±3.3	9.4±1.9	38.5±3.4	4.0±0.5	54.3±3.1	3.2±0.3	78.3±1.2	21.7±1.2
62	9.9±0.1	6.5±1.0	5.2±0.9	56.5±7.5	9.9±2.5	37.9±3.5	3.6±0.5	55.2±3.4	3.3±0.3	78.2±0.8	21.8±0.8
63	10.0±0.3	6.2±1.0	5.0±1.0	53.4±4.1	9.6±2.2	37.5±3.3	3.5±0.6	55.7±3.3	3.2±0.3	76.3±0.9	23.7±0.9
64	10.0±0.2	5.8±0.9	4.7±0.9	49.5±2.9	9.6±2.2	36.5±3.4	3.5±0.6	56.9±3.6	3.1±0.3	76.0±2.2	24.0±2.2
65	9.9±0.3	6.3±0.2	5.2±0.3	52.2±4.6	10.2±1.4	33.2±2.7	3.2±0.5	60.3±2.4	3.3±0.3	76.2±2.4	23.8±2.4

66	10.3±0.5	6.3±0.1	5.1±0.2	49.0±2.3	10.6±0.9	33.6±2.7	3.0±0.6	60.3±2.3	3.2±0.3	75.6±2.6	24.4±2.6
67	11.0±0.3	6.2±0.1	5.1±0.2	46.1±6.3	11.4±1.9	33.3±2.5	2.9±0.4	61.0±2.2	2.8±0.2	74.4±3.0	25.6±3.0
68	11.2±0.9	5.9±0.3	4.9±0.4	41.0±3.0	12.2±1.8	31.4±3.1	2.7±0.5	63.2±3.0	2.7±0.1	73.3±2.7	26.7±2.7
69	12.3±1.8	6.4±0.7	5.3±0.7	42.0±3.8	13.0±2.8	31.0±1.9	2.6±0.5	63.9±1.8	2.5±0.2	71.4±2.5	28.6±2.5
70	14.8±0.7	16.0±1.8	13.0±1.6	78.9±3.6	16.4±1.3	37.2±1.0	2.3±0.2	57.9±1.0	2.6±0.1	76.9±3.1	23.1±3.1
71	15.4±0.4	59.5±4.4	47.6±3.7	304.3±21.9	15.7±0.7	41.9±0.2	2.2±0.1	52.6±0.1	3.3±0.2	82.9±1.6	17.1±1.6
72	15.7±0.3	64.5±0.9	51.2±1.0	359.8±7.1	14.2±0.3	42.7±0.8	2.0±0.04	51.9±0.5	3.4±0.4	83.9±1.2	16.1±1.2
73	13.7±0.9	26.8±0.6	21.0±0.4	227.4±6.8	9.3±0.4	42.1±1.3	2.4±0.2	52.4±0.8	3.2±0.5	80.8±0.7	19.2±0.7
74	10.0±1.1	15.9±0.3	12.7±0.2	166.0±12.4	7.7±0.6	39.1±0.9	3.5±0.3	54.2±0.6	3.2±0.6	79.3±0.8	20.7±0.8
75	9.4±0.5	9.5±0.4	8.1±0.2	109.4±5.4	7.5±0.4	31.6±2.4	3.4±0.3	61.3±2.1	3.7±0.6	79.4±2.1	20.6±2.1
76	9.4±0.6	6.2±0.5	5.3±0.6	75.5±13.0	7.2±0.6	30.4±4.4	3.3±0.4	62.6±4.4	3.7±0.4	74.8±0.6	25.2±0.6
77	9.1±0.3	4.7±0.6	4.1±0.6	54.1±11.5	7.9±1.2	26.9±2.9	3.5±0.7	65.9±3.5	3.7±0.1	71.2±2.3	28.8±2.3
78	9.1±1.0	4.3±0.9	3.8±0.9	46.3±13.1	8.6±1.5	24.7±3.2	3.4±0.8	68.0±4.1	3.9±0.1	71.2±2.9	28.8±2.9
79	9.1±1.0	3.9±0.9	3.4±0.9	40.8±11.0	8.6±1.7	22.9±3.7	3.3±0.8	70.0±4.6	3.8±0.2	66.7±3.2	33.3±3.2
80	11.2±2.5	2.6±1.1	3.3±1.1	33.2±4.5	9.5±2.3	19.4±2.4	3.3±1.0	73.6±3.6	3.6±0.3	62.5±5.0	37.5±5.0

a = PA concentration in each fraction following HPLC/phloroglucinolysis determined by the summation of individual subunit concentrations determined using published conversion factors (*24*)

b = PA concentration in each fraction following HPLC/phloroglucinolysis determined by the summation of individual subunit concentrations calculated as catechin equivalents

c = PA concentration in each fraction determined by absorbance at 280nm on a UV-Vis spectrophotometer before phloroglucinolysis

abbrev. Epigall = Epigallocatechin, Cat = Catechin, Ecat = Epicatechin, Ecatgall = Epicatechin gallate, n.d. = not detected.

APPENDIX 2.

PAPER: REVIEW: CONDENSED TANNIN AND CELL WALL INTERACTIONS AND THEIR IMPACT ON TANNIN EXTRACTABILITY INTO WINE

Hanlin, R.L., Hrmova, M., Harbertson, J.F. & Downey, M.O. (2010). Review: Condensed tannin and grape cell wall interactions and their impact on tannin extractability into wine.
Australian Journal of Grape and Wine Research, v. 16 (1), pp. 173-188

APPENDIX 3.

PAPER: COMPARISON OF ETHANOL AND ACETONE MIXTURES FOR EXTRACTION OF CONDENSED TANNIN FROM GRAPE SKIN

Downey, M.O. & Hanlin, R.L. (2010). Comparison of ethanol and acetone mixtures for extraction of condensed tannin from grape skin.
South African Journal of Enology and Viticulture, v. 31 (2), pp. 154-159